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EXCITATORY AND INHIBITORY CONTROL OF INHERENT CONTRACTIONS IN THE SEA ANEMONE *CALLIACTIS PARASITICA*

By I. D. McFARLANE

Gatty Marine Laboratory and Department of Natural History, University of St Andrews, Scotland

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INTRODUCTION

The shape of the column of a sea anemone depends partly on the relative state of contraction of two antagonistic muscle groups in the body wall endoderm. These muscles, the circulars and the longitudinals (parietals), are attached to the mesogloea and act against the hydrostatic pressure in the coelenteron. The circulars form a continuous muscle sheet whereas the parietals lie only where mesenteries insert onto the body wall. Both muscles give slow contractions that can be either symmetrical or local. Column extension and column shortening result from symmetrical contractions; peristaltic waves and column bending result from local contractions, although in some species column bending may be due to contraction of parieto-basilar muscles. Although within wide limits the muscles have no fixed resting length, the body shape may remain constant for long periods. The circular and parietal muscles are not, however, at rest. In *Metridium senile* continual activity of the column muscles maintains the average state of tone that balances the hydrostatic pressure (Batham & Pantin, 1950*a*). Both *M. senile* and *Calliactis parasitica* show occasional slow spontaneous contractions involving several muscle groups (Batham & Pantin, 1950*b*; Needler & Ross, 1958); the contraction cycles differ in detail between the two species but each involves parietal and circular muscle activity. Batham & Pantin (1954) proposed, from kymograph studies of *Metridium*, that spontaneous contractions follow low-frequency bursts of pulses in the through-conducting nerve net. Such bursts can be directly recorded from isolated preparations of *Calliactis parasitica* and are followed by parietal and circular muscle contractions (McFarlane, 1973*a*, *b*). Batham and Pantin also suggested, however, that the nerve net is not necessarily directly responsible for exciting the observed contractions but that it may act to co-ordinate the natural inherent activity of the separate muscle groups involved. The nature of the control of parietal and circular muscle contractions is here reviewed in the light of the recent demonstration of multiple conduction systems in sea anemones (McFarlane, 1969*a*, 1973*c*).

There are three known conduction systems in *C. parasitica*; these are the nerve net and two slow-conducting systems, the SS1 and SS2, possibly non-nervous and apparently located in the ectoderm and endoderm respectively (McFarlane, 1969*a*). Spontaneous SS2 activity has been recorded from isolated preparations and intact animals (McFarlane, 1973*a*, *b*) but no behavioural correlate was described. The present work relates circular and parietal muscle contractions to electrical activity in

the nerve net and the SS2, in an attempt to elucidate the control systems involved in the spontaneous contraction cycle and in the maintenance of body shape.

A maintained body shape results partly from the properties of the mesogloea and the hydrostatic skeleton, so that after a spontaneous contraction there will be a natural tendency for the muscles to return to their original length. This is not, however, a permanently fixed length; at times the activity of one muscle group may predominate and this is reflected in the adoption of a new resting shape. As Pantin (1965) pointed out, the ability to maintain a fixed shape may also depend on control by some proprioceptive mechanism. This would be an internal system capable of detecting and correcting deviations from some set position. If comparable to such systems in higher animals this might be expected to involve both excitatory and inhibitory control of the muscle groups. Batham & Pantin (1954) gave evidence of reciprocal inhibition between parietal and circular muscles in *Metridium senile* but did not suggest how this was co-ordinated. To date, the only direct evidence for inhibition is that of Ewer (1960) who showed that the nerve net has both excitatory and inhibitory actions on isolated circular muscle rings cut from the body wall of *Calliactis parasitica*.

The present work indicates that the spontaneous contraction cycle results from the controlling actions of the nerve net and the SS2 on an inherent muscular activity. Evidence is given here that the SS2 inhibits contractions of circular and parietal muscles. Ewer's results remain valid, however, as nerve-net activity also seems to inhibit circular muscles. It is suggested that the SS2 functions as part of a proprioceptive system which monitors the activity of these antagonistic muscles and controls contractions by inhibition. The two slow-conduction systems thus have similar actions, as it has been previously shown that the SS1 in *Tealia felina* seems to inhibit contractile activity of the ectodermal muscles of the oral disc (McFarlane & Lawn, 1972).

MATERIALS AND METHODS

This study used specimens of *Calliactis parasitica*, with an expanded oral disc diameter of 3–5 cm, obtained from the Marine Laboratory, Plymouth. Apparatus and recording techniques are as previously described. Double circular muscle and double parietal muscle preparations were obtained as described before (McFarlane, 1973*b*). Circular muscle preparations consisted of two partly isolated column rings, one cut from the pedal region, the other from the mid-column region. Parietal muscle preparations consisted of two partly isolated strips of column. The rings or strips were left connected to a portion of the body bearing tentacles. Preparations were used from 1 to 6 days after operation; beyond this age they deteriorate rapidly and the rings or strips may break under load. Electrical activity was monitored by two suction electrodes attached to tentacles. Contractions were recorded with light isotonic levers connected to photo-electric transducers. Contractions and accompanying electrical activity were displayed on a pen recorder. Note that the pulses shown in the figures are not direct recordings; this is because pulses are not readily identifiable at the slow paper speed required to display the slow contractions. Consequently, electrical activity was monitored on an oscilloscope at a sweep speed of 200 msec/cm and when pulses were identified their occurrence was marked on the record. Activity was monitored from all three conduction systems (the nerve net, SS1 and SS2) but SS1 activity is omitted

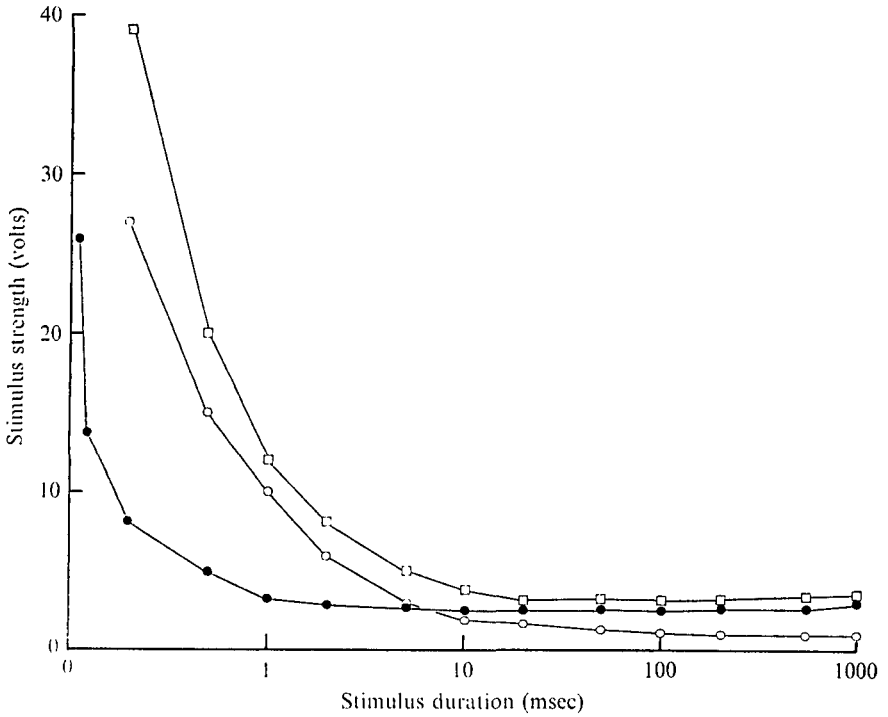

Fig. 1. Stimulus strength/duration curves for the three conduction systems in *Calliactis parasitica* – nerve net (●), SS2 (○), SS1 (□). The stimulus threshold for each system is plotted over a wide range of stimulus durations. Stimulating and recording electrodes attached to tentacles. Note that the nerve net and SS2 curves intersect.

from most of the figures because usually very few SS1 pulses were recorded and the system does not seem to be directly involved with the endodermal events studied here.

Recordings were made from tentacles as this is the only region where activity in all three conduction systems can be monitored simultaneously. The integrity of conduction paths was checked by stimulating the endodermal face of each ring or strip; evoked SS2 and nerve-net pulses indicate an intact conduction path up the column. The recorded spontaneous pulses might, however, originate close to the recording electrodes, and if the conduction systems are polarized these pulses may not be reaching the muscles. Pulses cannot be recorded from electrodes attached to the region of the muscles, but conduction down the column is inferred from experiments where an animal was bisected, leaving only a narrow bridge of pedal disc tissue to connect the two halves. SS2 and nerve-net pulses elicited by tentacle stimulation of one half are recorded from tentacles on the other half. The structural basis of the SS2 is not known but there is histological evidence for a nerve net in the region of these muscles in *Metridium senile* (Batham, Pantin & Robson, 1960). It is here assumed that all recorded nerve-net and SS2 pulses reach the vicinity of the muscles.

Essential to the study was the development of a technique for stimulating the SS2 alone. At normal stimulus-pulse durations the threshold of the SS2 is higher than that of the nerve net, so it is only possible to stimulate either the nerve net alone or both systems together. This is true whether the stimulating electrode is attached to tentacles,

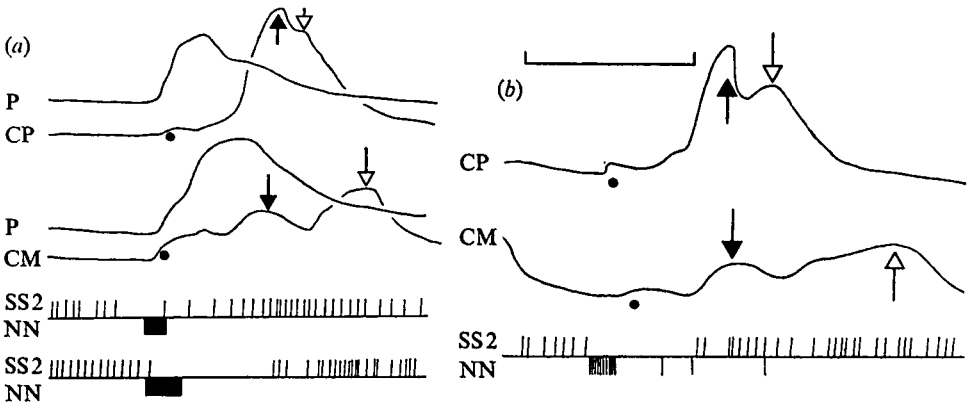

Fig. 2. (*a*) Spontaneous contractions and associated electrical activity from a double circular muscle preparation (CP, pedal ring; CM, mid-column ring) and from a double parietal muscle preparation (P). The records are superimposed to show the sequence of contractions comprising the spontaneous contraction cycle. Upper electrical record is from the parietal preparation, lower from the circular preparation; SS2 pulses shown above the line, nerve net (NN) bursts shown as solid blocks below the line. The symmetrical parietal muscle contraction appears first, followed by the symmetrical circular muscle contraction (closed arrows) and finally the local circular muscle contraction (open arrows). (*b*) Symmetrical and local contractions following electrical stimulation of the nerve net (10 shocks at 1 every 10 sec) in a double circular muscle preparation. Note the reduction in SS2 activity accompanying nerve-net activity in both spontaneous and evoked records. Dots show deflexions on the circular muscle record that are probably caused by the parietal muscle contraction. Time scale = 5 min.

oral disc, or mesenteries. The SS1 can be stimulated alone by attaching the stimulating electrode to a thin flap cut in the ectodermal surface of the column (McFarlane, 1969*b*), but attempts to isolate the nerve net and the SS2 by cutting endodermal flaps proved unsuccessful. When, however, stimulus strength/duration curves are plotted for tentacle stimulation of the three conduction systems it is seen that the nerve net and the SS2 curves intersect so that at long stimulus durations the SS2 has the lowest threshold (Fig. 1). In practice all stimuli were applied to tentacles; at 1 msec duration to excite the nerve net only and at 200 msec duration to excite the SS2 only. The nerve net and SS2 were stimulated simultaneously by 1 msec duration shocks at a voltage just below SS1 threshold.

RESULTS

Terminology. The term 'spontaneous' throughout refers to contractions that follow a nerve-net burst whereas 'inherent' refers to contractions that appear in the absence of nerve-net activity. 'Symmetrical' refers to contractions that occur more or less simultaneously in an entire muscle system; 'local' refers to contractions displayed by only a restricted portion of a muscle system at any one time.

Spontaneous contractions and electrical activity

This section describes the spontaneous contractile activity of isolated preparations and relates observed contraction patterns to recorded electrical activity. Both the nerve net and the SS2 seem to be involved in the control of spontaneous contractions.

Some isolated preparations show contractions which only follow nerve net bursts;

the contractions can be directly related to behaviour of the intact animal. At intervals the intact animal shows a spontaneous contraction sequence involving tentacle twitching, a slow submarginal contraction, a slow symmetrical parietal muscle contraction, a large slow sphincter muscle contraction, a slow symmetrical circular muscle contraction, and finally a peristaltic wave that passes up the column from the pedal disc (Needler & Ross, 1958). Considering here just the parietal and circular muscle contributions, a similar contraction sequence follows a spontaneous burst of nerve-net pulses in isolated preparations. Bursts usually consist of 10–15 pulses with a pulse interval of 4–10 sec (McFarlane, 1973*b*). Fig. 2(*a*) shows spontaneous contractions of a double parietal muscle preparation and of a double circular muscle preparation; the records are superimposed to show the normal spontaneous contraction sequence. The start of the nerve-net burst is shortly followed by almost simultaneous contraction of both parietal muscle strips; this is taken to be the symmetrical parietal muscle contraction shown by the intact animal. Parietal muscle relaxation is accompanied by a symmetrical contraction of the circular muscles. Next, there is a local contraction of the circular muscles, appearing first in the pedal ring. This probably represents the passage of the peristaltic wave in the intact animal. As has also been noted in *Metridium senile* (Batham & Pantin, 1950*b*), the circular muscle contractions show a long latency; here the symmetrical contractions begin about 100 sec after the start of the nerve-net burst. The early rise in the circular muscle record (marked by dots) is not seen in all preparations and is taken to be an artifact due to mechanical interference from the parietal muscle contraction.

Spontaneous contractions usually occur at intervals of 10–20 min in preparations, but at much longer intervals in intact animals (Needler & Ross, 1958; McFarlane, 1973*b*). This may be related to the load on the muscles, as contraction interval is decreased when an intact animal is connected to a kymograph lever (Needler & Ross, 1958).

Electrical stimulation of the nerve net, at a pulse number and pulse frequency within the range seen in spontaneous bursts, is followed by a contraction pattern identical to the spontaneous sequence. Fig. 2(*b*) shows circular muscle contractions following 10 shocks at a stimulus interval of 10 sec; both symmetrical and local contraction components are obvious. Symmetrical parietal muscle contractions can be elicited in the same way.

All spontaneous nerve-net bursts are followed by parietal and circular muscle contraction; but a simple model for contraction control cannot follow because, as described below, contractions of either muscle group sometimes occur in the absence of recorded nerve-net activity. A model must also explain the long latency of circular muscle contraction and the origin of the two types of circular muscle contraction. In addition, account must be taken of the possible function of the SS2.

Recordings from half-animal preparations showed a reciprocal relationship between nerve-net and SS2 activity; during a nerve-net burst few or no SS2 pulses are seen; following the burst the SS2 pulse frequency rises rapidly and then slowly declines up to the start of the subsequent nerve-net burst (McFarlane, 1973*a*). The reduction in SS2 activity during the nerve-net burst will be termed the SS2 quiet period. Fig. 2(*a*, *b*) shows SS2 activity recorded during spontaneous and evoked contractions. In both cases an SS2 quiet period accompanies nerve-net activity, indicating a link

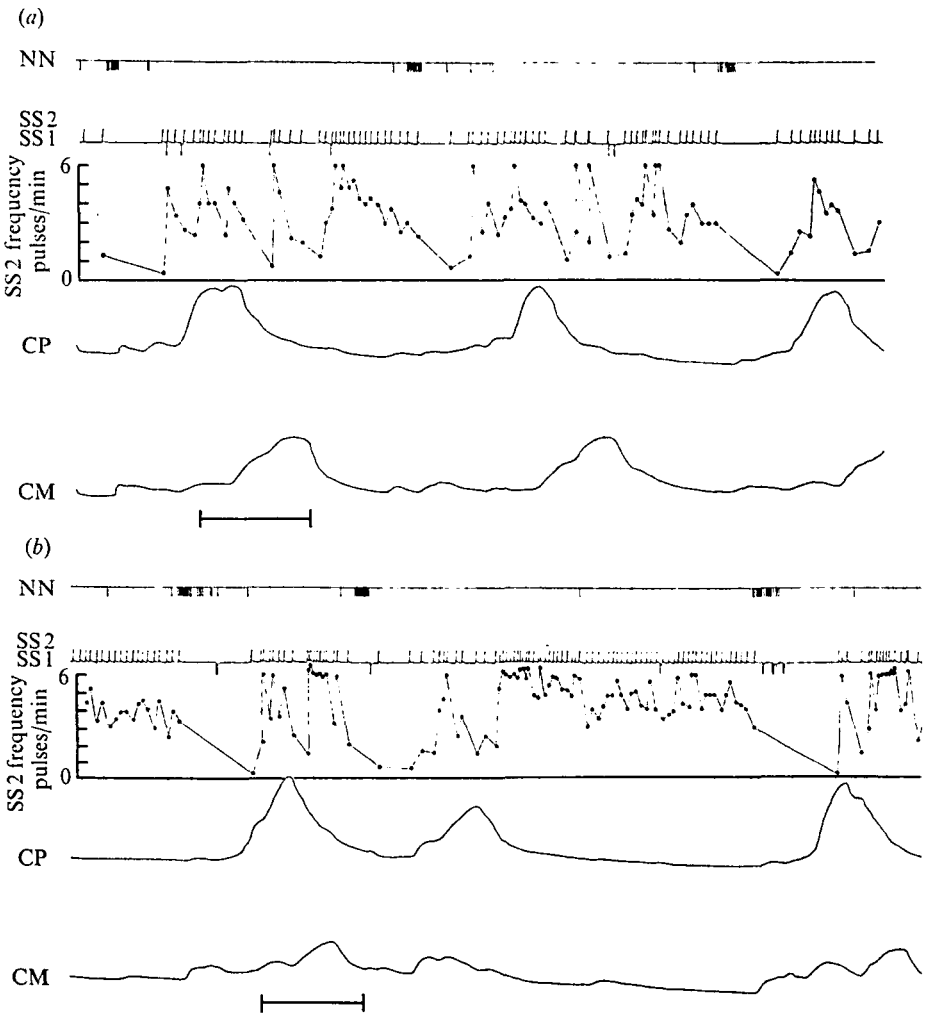

Fig. 3. Long-term records of contractions and associated electrical activity from 3-day-old double circular muscle preparation. Electrical activity shown from all three conduction systems, nerve net (NN), SS1 and SS2. SS2 activity is also shown plotted as instantaneous pulse frequency against time. CP, pedal ring; CM, mid-column ring. (*a*) Contractions following three nerve-net bursts with interburst intervals of 13 min. (*b*) Contractions following bursts separated by a short interval (9 min) succeeded by a long interval (20 min). The SS2 activity stays at a high frequency during the long interburst interval. Time scale = 5 min.

between the SS2 and either the nerve-net pulses or the associated contractions. In the half-animal preparations the mean SS2 pulse frequency was 1·2–2·5 pulses/min, with a peak frequency of about 7·5 pulses/min (McFarlane, 1973*a*). In the preparations studied here the frequencies are similar, for example, in Fig. 3(*a*) the mean is 2·3 pulses/min and the peak is 7 pulses/min.

Maintained SS2 pulse frequencies greater than about 4 pulses/min are usually associated with muscular inactivity. Fig. 3 shows contractions and electrical activity of a double circular muscle preparation. In Fig. 3(*a*) the interval between nerve-net bursts (the interburst interval) is fairly constant at about 13 min. Note that occasional nerve net pulses are seen outwith bursts. The SS2 activity is shown plotted as pulses

per min. The SS2 pulse frequency is low during nerve-net bursts and high during the interburst interval. In half-animal preparations the rise in SS2 frequency comes almost immediately after the burst and the decline from the peak frequency tends to be smooth. The changes in SS2 frequency differ, however, in isolated preparations; as discussed later, this may be because the muscles are not acting against a hydrostatic skeleton. There is often a noticeable lag of 1–2 min between the end of the burst and the rise in SS2 frequency. Following the rise there are often fluctuations in frequency before the gradual decline that is followed by the next nerve-net burst. The contractions do not seem to directly cause the changes in frequency as this may be high during periods of muscle activity or inactivity. These are not, however, truly isolated preparations as they contain other muscle groups, and the origin of the SS2 pulses may be related to events occurring elsewhere in the preparation. In Fig. 3(*b*) a short interburst interval (9 min) is followed by a long interburst interval (20 min) during which the SS2 activity does not show the usual decline in frequency and the muscle rings remain inactive. The SS2 pulses may cause this inactivity either by directly inhibiting the muscles or by prolonging the interburst interval. The observed events are not related to the preceding short interburst period as long intervals sometimes follow intervals of average duration.

A clear association between SS2 activity and contraction is observed in the relationship between the duration of the SS2 quiet period and the size of the symmetrical component of the circular muscle contractions. For a single preparation, the symmetrical component shows considerable variation in size following successive nerve-net bursts, whereas the size of the local contraction remains fairly constant. Fig. 4(*a*) shows three spontaneous contractions of a double circular muscle preparation. The SS2 quiet period always starts before the symmetrical contraction appears and ends before the peak of contraction is reached. Results from the same preparation are shown graphically in Fig. 4(*b*); this plots the height of the symmetrical pedal ring contraction against the duration of the SS2 quiet period. Clearly, the longer the quiet period the larger the contraction. The size of the symmetrical contraction seems unrelated to nerve-net parameters such as interburst interval, pulse number, or pulse frequency. As the resumption of SS2 activity precedes the peak of contraction these results suggest that the SS2 can inhibit development of symmetrical circular muscle contractions during the spontaneous contraction sequence.

As preparations age, spontaneous contractions tend to last longer and it generally becomes difficult to distinguish symmetrical and local circular muscle contractions. Fig. 5 shows circular muscle contractions following nerve-net bursts in a 5-day-old preparation. Although the interburst intervals are within the range seen in younger preparations, the muscles spend less time in a quiescent state. This is due both to an earlier onset of contraction and to a reduced rate of relaxation; there may also be additional contractions during the interburst interval. The observed changes in muscular activity may be related to a changed distribution of SS2 activity, usually involving both a reduced mean frequency and the absence of the normal rise in frequency following the nerve-net burst. The earlier onset of contraction, however, may relate to the increasingly excitatory effect of nerve-net pulses as circular muscle preparations age; this is described in the section dealing with electrical stimulation. The results again suggest that the SS2 is inhibitory, the extended contractions being

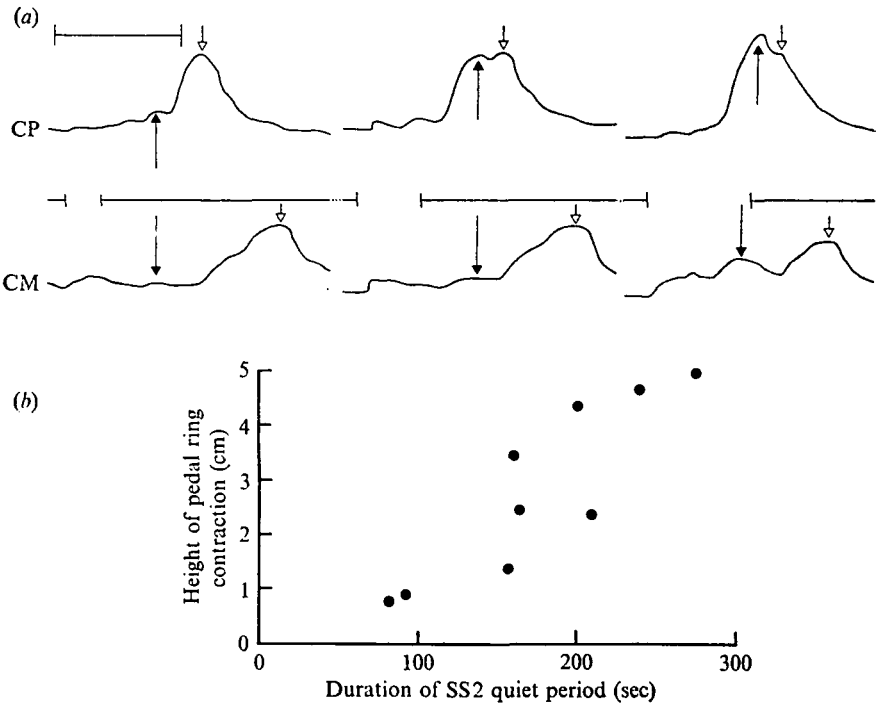

Fig. 4. Relationship between size of symmetrical circular muscle contraction and duration of SS2 quiet period. (*a*) Records of circular muscle contractions following nerve-net bursts (symbols as in Fig. 2). The breaks in the line between the upper and lower record show the duration of the SS2 quiet period accompanying the nerve-net burst. The size of the local contraction remains fairly constant in successive spontaneous contractions but the symmetrical contraction size varies, apparently according to the duration of the SS2 quiet period. Time scale = 5 min. (*b*) Plot of the size of the pedal ring contraction (cm measured from record) against the duration of the quiet period. All points were obtained from the preparation shown in (*a*).

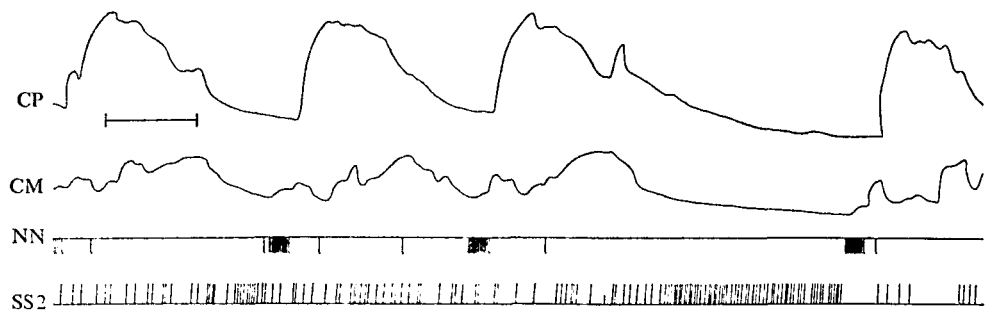

Fig. 5. Contractions and associated electrical activity from a 5-day-old circular muscle preparation. Symbols are as before. Here the muscles relax more slowly than in younger preparations and small additional contractions sometimes occur during the interburst interval. This may be related to a changed distribution of SS2 activity, suggesting that the SS2 may normally function to cut off spontaneous contractions. Time scale = 5 min.

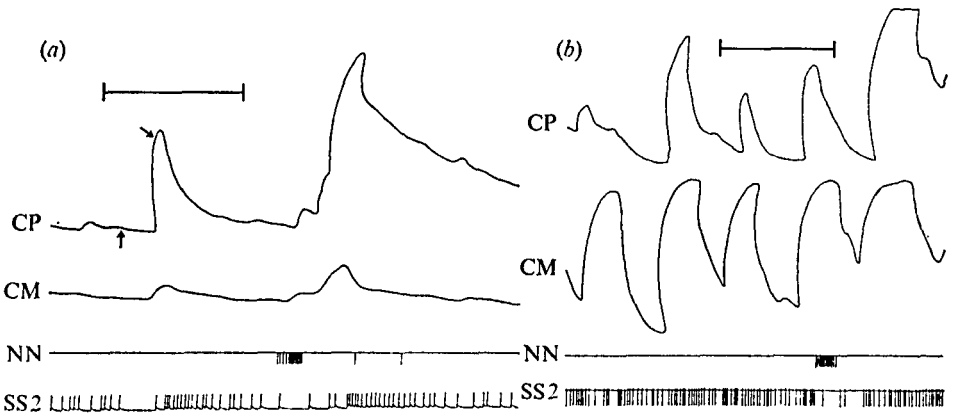

Fig. 6. (*a*) Circular muscle contractions in the absence of nerve-net activity. A symmetrical contraction seems here to follow a period of reduced SS2 activity, the beginning and end of which are indicated on the contraction record by arrows. This contraction is followed by a larger contraction associated with a nerve-net burst. (*b*) Rhythmic contractions seen in a 7-day-old circular muscle preparation. This inherent activity appears even though the SS2 pulse frequency is high. The recorded pulses are probably not, however, reaching the muscles because of conduction failure in the region of the rings. Time scale = 10 min.

due to a decreased amount of inhibition. Fig. 5 also shows a period of high-frequency SS2 activity during which the muscles remained inactive. Long-lasting spontaneous contractions are also seen as parietal muscle preparations age.

Inherent contractions and electrical activity

In some preparations nerve-net bursts may be present and followed by spontaneous contraction; but other contractions, termed inherent, are seen without preceding nerve-net activity. This is not simply a case of failure to record nerve-net pulses as they can be evoked by electrical stimulation. The appearance of inherent contractions may be related to ageing, as preparations that for the first 2 or 3 days give contractions only after nerve-net bursts may subsequently show inherent contractions. Other preparations, however, show from the outset contractions that are not linked to nerve-net bursts.

Some preparations, usually those with a mean SS2 pulse interval greater than 60 sec, give large rhythmic contractions, often at intervals of only 2·5–4 min. These contractions can occur even in the total absence of recorded nerve-net activity. Most other preparations give occasional inherent contractions at irregular intervals; these preparations usually show a mean SS2 pulse interval of 15–60 sec. When contractions do occur they seem to follow periods of reduced SS2 activity (Fig. 6*a*). All preparations may also show occasional single nerve-net pulses or bursts, the latter being followed by contractions and accompanied by a reduction in SS2 activity (Fig. 6*a*). Inherent contractions tend to be symmetrical in the type of preparation shown in Fig. 6(*a*) but are not always symmetrical in preparations where the SS2 frequency is very low.

In preparations more than 6 days old, regular rhythmic contractions, similar in appearance to those seen in preparations where SS2 frequency is very low, may appear in the absence of nerve-net activity but in the presence of high-frequency SS2 activity (Fig. 6*b*). This apparently anomalous result seems associated with conduction failure

and usually occurs just before the rings or strips break. Electrical stimulation of such strips or rings fails to elicit pulses that reach the tentacles, suggesting that the recorded pulses are not reaching the muscles. These contractions may represent the basic inherent muscular activity, unmodified by SS2 or nerve-net activity. The cause and origin of the observed high-frequency SS2 pulses is not known.

It seems then that control of muscular activity involves the nerve net and SS2 acting, not on quiescent muscles, but on a background of inherent activity. The existence of inherent activity has already been proposed in *Metridium senile* by Batham & Pantin (1950*b*). This activity appears here as regular, unsynchronized contractions at intervals of 2·5–4 min when the SS2 frequency is very low. The contractions associated with nerve-net bursts, however, occur at intervals of 10–20 min. This implies that in preparations showing only spontaneous contractions the inherent activity is either not present or is being damped by an inhibitory mechanism. The results presented above suggest that the SS2 constitutes this mechanism, so that when SS2 pulse frequency is high the inherent contractions are reduced or abolished. The above observational experiments cannot, however, eliminate the possibility that some other system is causing both the observed inhibition of the muscles and the changes in SS2 frequency.

Electrical stimulation of conduction systems in isolated preparations

Most of the preparations used for stimulation experiments showed a low SS2 pulse frequency and contractions in the absence of nerve-net bursts. Thus this section describes the actions of the conduction systems on inherent muscular activity.

Although electrical stimulation allows a more direct confirmation of the actions of the nerve net and SS2, it presents certain interpretative difficulties. First, there is the problem of conduction-system interaction. Stimulation of one conduction system is often associated with a change in the spontaneous activity of the other. For example, low-frequency nerve-net stimulation is usually accompanied by a decrease in SS2 pulse frequency, so it is not always obvious whether the observed muscle responses are due to the elicited nerve-net pulses or to the reduction in SS2 activity. Secondly, stimulation of the nerve net occasionally triggers partial or complete nerve-net bursts (McFarlane, 1973*b*). Clearly, continuous monitoring of electrical activity is essential in order to interpret the results of electrical stimulation. Thirdly, as these are not completely isolated preparations, there may be interaction between muscle systems. For example, stretch might modify or trigger contractions and thus link the action of these antagonistic muscles. Although this may be true in the intact animal where the hydrostatic skeleton is present, its importance cannot be easily determined in these preparations.

Electrical stimulation of the SS2 results in abolition of inherent contractions in parietal muscle preparations (Fig. 7*a*). In the example shown the stimulus interval was 10 sec but inhibition can often be detected at stimulus intervals as long as 60 sec. The delay in the onset of inhibition seems dependent on when stimulation is applied; if begun at the start of a spontaneous contraction this may continue; if started at the peak of contraction an increased rate of relaxation is often detected and usually no further contractions appear. Small contractions may occur during stimulation but the overall effect is abolition of inherent activity and a gradual increase in length. Cessation

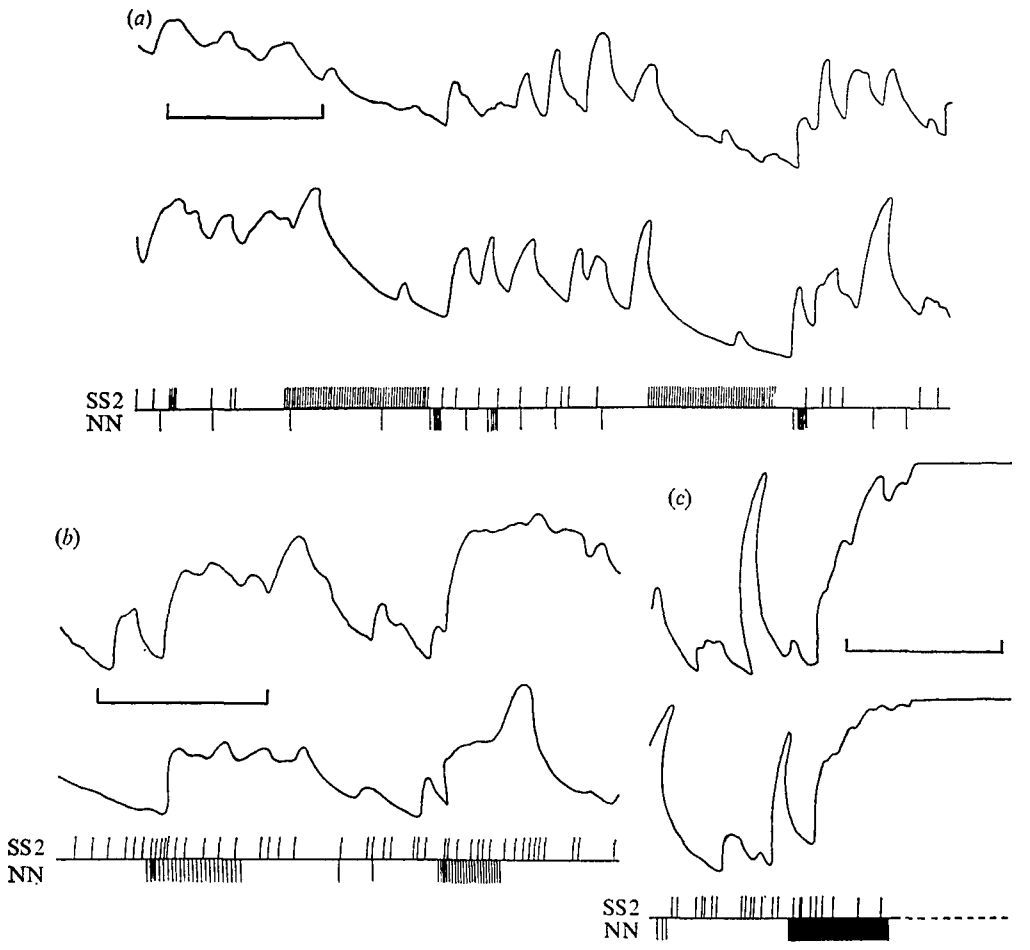

Fig. 7. (*a*) Effect of electrical stimulation of SS2, at 1 shock every 10 sec, on inherent activity of parietal muscle preparation. SS2 stimulation is accompanied by inhibition of inherent activity. End of stimulation is followed by a symmetrical contraction. (*b*, *c*) Effect of nerve-net stimulation on parietal muscle preparation. Three stimulus series are shown, with stimulus intervals of 15 sec, 10 sec, 5 sec. Time scale = 10 min.

of stimulation is always followed, after a delay of 50–60 sec, by contraction of both strips. One strip usually starts contracting a few seconds before the other and maintains this leadership after each period of SS2 stimulation. Following the symmetrical contraction, the normal inherent rhythm is resumed and the overall muscle length decreases though the original mean level is not always reached. Nerve-net activity, generally in the form of a single short burst, may appear following SS2 stimulation, but the presence of bursts is not essential for the development of symmetrical contractions. Bursts may appear before or during contraction.

Stimulation of the nerve net in parietal muscle preparations is always accompanied by contraction of both strips (Fig. 7*b*, *c*). The contraction starts after about 6 or 7 evoked pulses. At low stimulus frequencies the inherent activity can be seen superimposed on a raised tone level. At higher frequencies there is a larger and faster

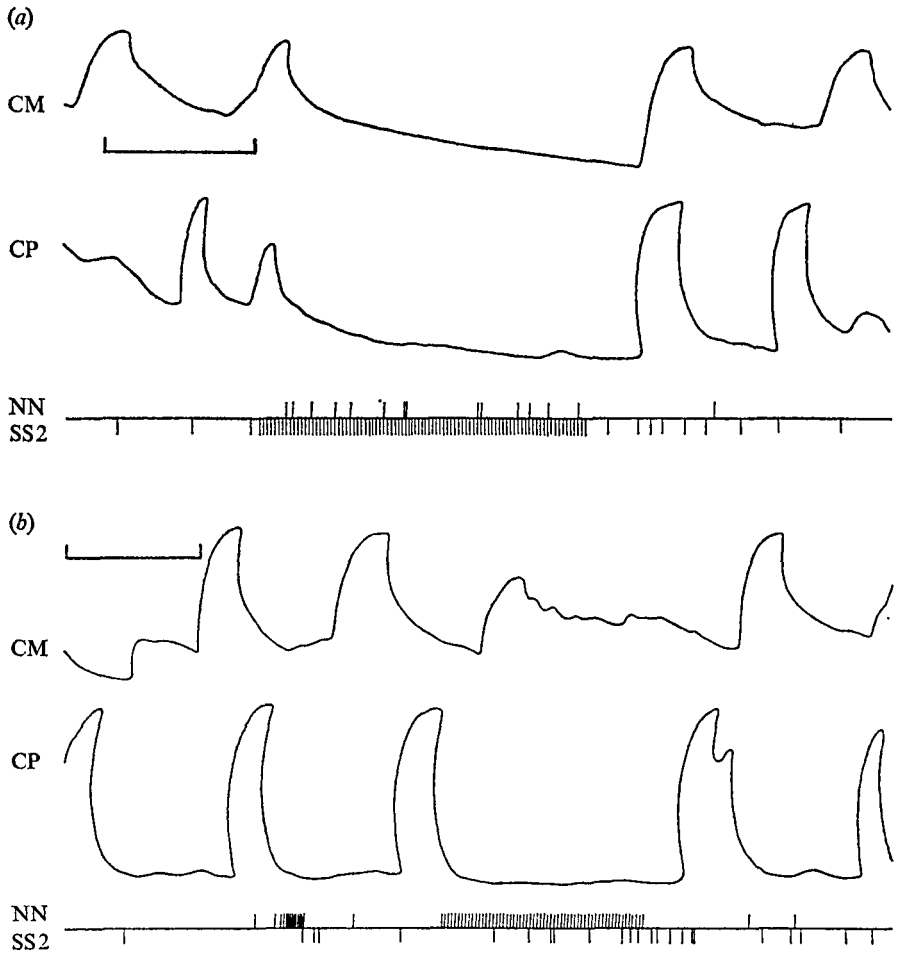

Fig. 8. (*a*) Effect of electrical stimulation of SS2, at 1 shock every 10 sec, on circular muscle preparation. Stimulation is accompanied by inhibition of both rings. The end of stimulation is followed by a symmetrical contraction. (*b*) Effect of nerve-net stimulation (1 shock every 10 sec) on the same preparation. The pedal ring is inhibited but the mid-column ring shortens. Stimulation is followed by a local contraction appearing first in the pedal ring. Time scale = 10 min.

contraction but the inherent activity is often still apparent (Fig. 7*c*). Maintained stimulation results in maintained contraction. Recovery after stimulation is usually slow. When relaxation is rapid it is accompanied by SS2 activity with pulse intervals less than 15 sec.

Stimulation of the SS2 in double circular muscle preparations results in inhibition of inherent contractions (Fig. 8*a*). Again there is a short delay before the action is obvious, inhibition is maintained for the duration of stimulation, and an effect can sometimes be detected at stimulus intervals as long as 60 sec. Cessation of stimulation is followed by a large contraction appearing more or less simultaneously in both rings, but for circular muscle preparations this delay is about 180–250 sec. The inhibitory effect thus seems to be longer-lasting in circular muscle preparations than in parietal muscle preparations. After this contraction the typical inherent activity is resumed.

Again, stimulation may be followed by a nerve-net burst but here this results in an increased contraction delay and often in a modification of contractions so that they appear to be local rather than symmetrical.

Surprisingly, it was found that nerve-net stimulation also inhibits circular muscle preparations. This inhibitory action can, however, be distinguished from the SS2 action. Ewer's (1960) observation that low-frequency nerve-net stimulation has both excitatory and inhibitory effects on circular muscle rings was made before the demonstration of multiple conduction systems in sea anemones and it was suspected that his results might be re-interpreted on the basis of independent effects of the nerve net and SS2. The results, however, fully support his findings. Nerve-net stimulation is accompanied by inhibition of circular muscles (Fig. 8*b*). This is true for pedal rings of all ages; but, as Ewer showed, whilst this inhibition appears in fresh rings from other regions, it rapidly disappears as the preparation ages and an excitatory action becomes more and more obvious. In Fig. 8(*b*) the inhibitory action on the mid-column ring has decayed, and although activity is reduced it is not abolished. The excitatory component of stimulation is shown by a maintained shortening of the muscle. Cessation of stimulation is followed by local contraction, the pedal ring usually contracting first. The delay from the end of stimulation to the start of contraction is 150–200 sec for pedal rings and 300–400 sec for mid-column rings. This particular preparation is unusual in showing regular contractions at intervals of 10 min in the presence of a very low level of SS2 activity, and apparently unmodified by the nerve-net burst shown in the record. The contractions following low-frequency nerve-net stimulation are not here readily distinguished from a simple return to inherent activity, but such contractions are also seen in preparations showing little or no inherent activity.

Both conduction systems seem to inhibit circular muscles, but SS2 stimulation acts equally on all rings of all ages and is followed by symmetrical contraction, whereas nerve-net stimulation acts differently on different rings as they age, appears to be combined with an excitatory action, and is followed by local contraction. These differences suggest that the nerve net and SS2 are not sharing a common inhibitory pathway. The observed inhibitory effect of nerve-net stimulation is not due to the SS2 as SS2 activity usually decreases during nerve-net stimulation. The converse is also true; in Fig. 8(*a*) the level of nerve-net activity increases during SS2 stimulation, but this is unusual and normally there are no accompanying nerve-net pulses.

Low-frequency nerve-net stimulation applied immediately after a spontaneous burst can reduce, abolish, or at least delay the spontaneous contraction (Fig. 9*a*). In this case the inhibitory effect on the mid-column ring has decayed and this ring contracts. The pedal ring, however, is inhibited even though all other nerve-net bursts in this preparation were followed by large pedal ring contractions. Similarly, SS2 stimulation applied after a spontaneous nerve-net burst can reduce or abolish contractions in both rings. This is also true for SS2, but not nerve-net, stimulation following spontaneous nerve-net bursts in parietal preparations.

The excitatory effect of low-frequency nerve-net pulses on mid-column rings can be reduced but not abolished by SS2 stimulation. Fig. 9(*b*, *c*) shows the effect of stimulating first the nerve net alone, and then the nerve net and SS2 together, at a frequency of one pulse every 10 sec. In both cases the pedal ring is inhibited whereas the mid-column ring contracts, although this contraction is reduced when there is accompanying SS2

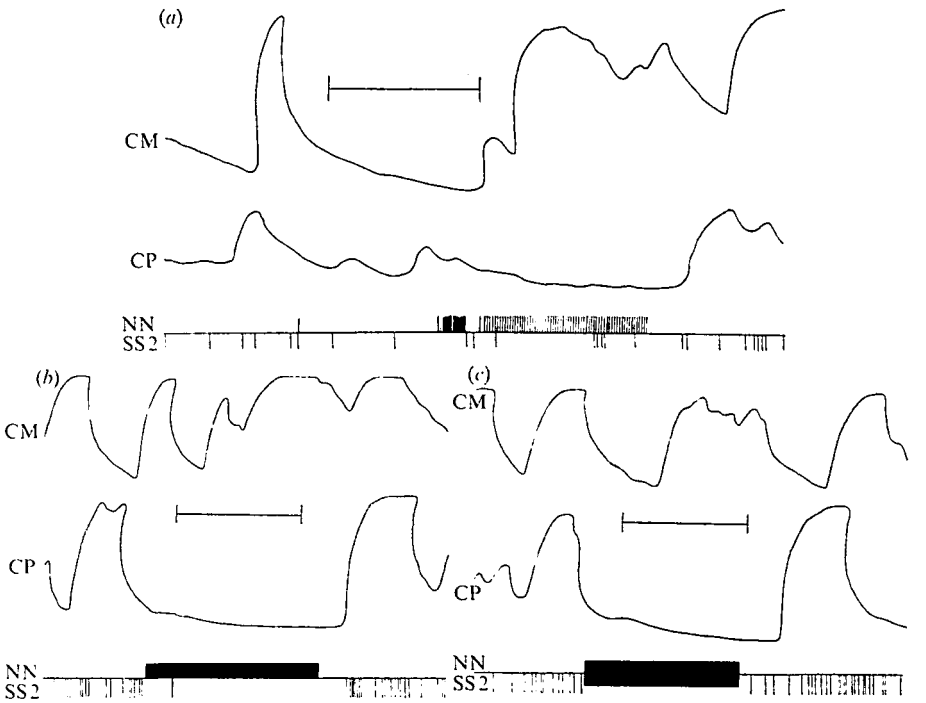

Fig. 9. (*a*) Low-frequency nerve-net stimulation applied immediately after a spontaneous burst in a 3-day-old circular muscle preparation results in abolition of the pedal contraction which normally follows the burst. The mid-column ring, however, contracts. (*b*) Stimulation of nerve net only and (*c*) nerve net and SS2, at 1 shock every 10 sec, in a circular muscle preparation. The SS2 activity reduces but does not prevent the mid-column ring contraction accompanying nerve-net stimulation. Time scale = 10 min.

activity. This implies that the mid-column ring contraction is not wholly due to a reduction in SS2 activity during nerve-net stimulation, but is related to an excitatory action of the nerve-net pulses.

Differences between nerve-net-mediated and SS2-mediated inhibition are further shown in experiments where a period of stimulation of one system is immediately followed by stimulation of the other. Fig. 10(*a*) shows the effect on a double circular muscle preparation of SS2 stimulation followed by nerve-net stimulation. Instead of the normal symmetrical contraction following SS2 stimulation, here only the mid-column ring contracted, showing again the differential action of nerve-net inhibition on different rings. Fig. 10(*b*) shows a younger preparation where an initial period of nerve-net stimulation inhibited both rings. Inhibition was maintained during a succeeding period of SS2 stimulation, but a return to nerve-net stimulation was accompanied by a symmetrical contraction. In general it seems that SS2 stimulation, at a sufficiently high frequency, can always prevent the development of the contractions that normally follow nerve-net stimulation, but the contractions following SS2 stimulation are not always abolished by evoked nerve-net pulses.

Parietal muscle preparations showing unsynchronized inherent contractions can be made to give regular symmetrical contractions by electrical stimulation of the SS2

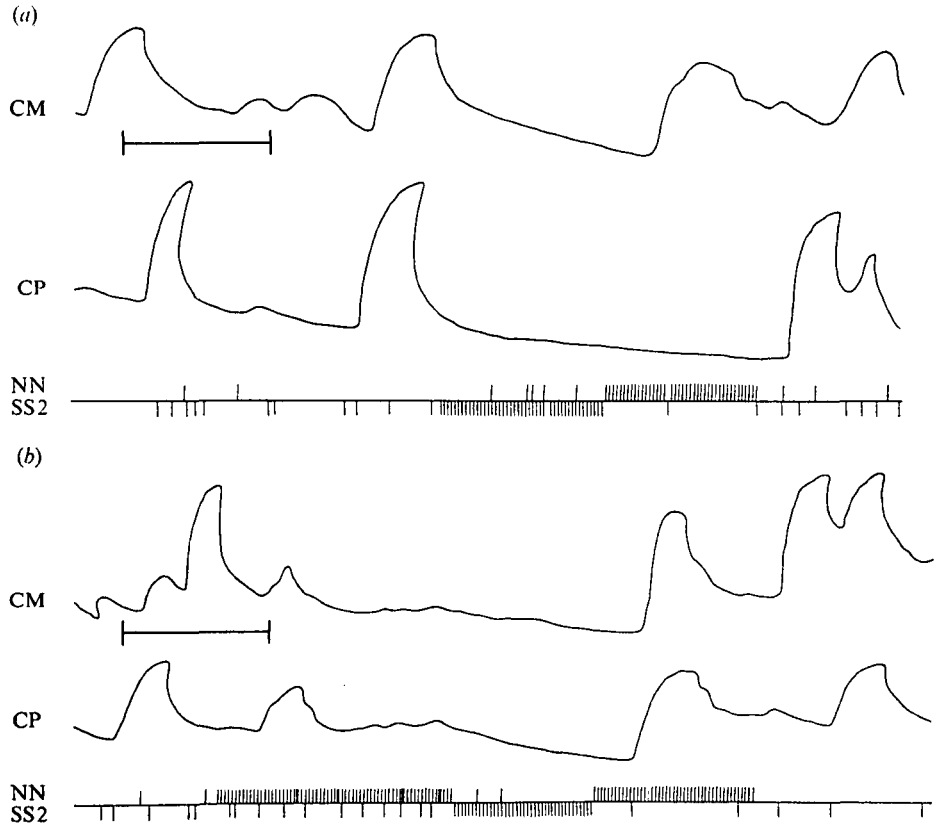

Fig. 10. Showing interaction between nerve-net-mediated and SS2-mediated inhibition of circular muscle preparations. (*a*) Period of low-frequency SS2 stimulation is normally followed by symmetrical contraction but nerve-net stimulation can prevent development of pedal ring contraction. (*b*) SS2 stimulation after a period of nerve-net stimulation prevents development of local contractions, but a return to nerve-net stimulation this time fails to stop symmetrical contractions. Time scale = 10 min.

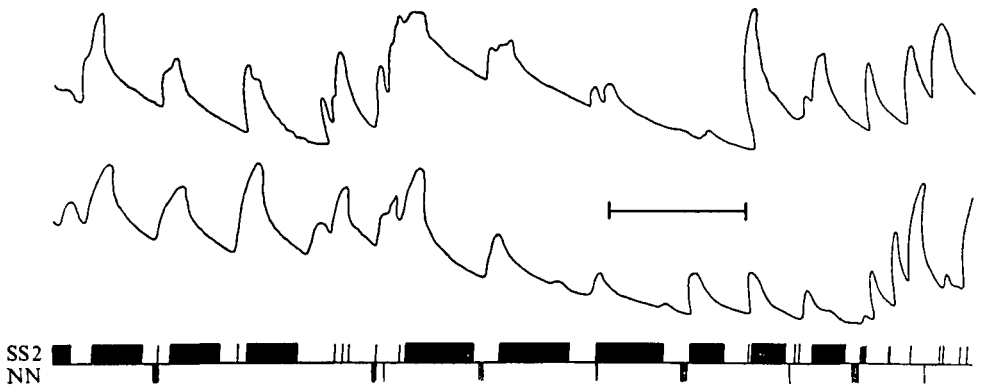

Fig. 11. A parietal muscle preparation giving local inherent contractions can be made to show regular symmetrical contractions by electrical stimulation of the SS2. This is effective over a wide range of imposed contraction intervals. Time scale = 10 min.

(Fig. 11). The SS2 was stimulated at a frequency of one shock every 7·5 sec, and an interval of 2 min was left between each stimulus series. As the stimulation inhibits the inherent activity and the 2 min break removes the inhibition, the preparation is driven to contract symmetrically at a contraction interval determined by the duration of the period of inhibition plus the duration of the break. The first three stimulus series shown were each 210 sec in duration, thus the interval between contractions is 330 sec. Stimulation was then stopped and the muscles rapidly returned to their original inherent rhythm. Further periods of stimulation drove the contractions first at intervals of 410 sec and then at intervals of 270 sec. Again, at the end of the record stimulation was stopped and the original activity returned. Although there are occasional short nerve-net bursts it is clear that contractions can be driven just by SS2 stimulation. Note, however, that contraction height varies at different contraction intervals. SS2 stimulation can also drive circular muscle preparations in the same way. Evoked nerve-net bursts can also be used to drive preparations, but this generally results in a gradual increase in tone level until contractions are no longer clear. This can be prevented by stimulating the SS2 during the interburst interval, in other words by simulating the pattern of electrical activity seen in preparations giving only spontaneous contractions.

Electrical stimulation of conduction systems in intact animals

Electrical stimulation of intact animals provides a dramatic demonstration of the actions of the conduction systems, but analysis of the observed shape changes is very difficult.

Stimulation of the SS2 at a frequency of one shock every 10 sec results in a clear change of shape (Fig. 12). The tentacles become limp and point downwards, the margin of the oral disc is folded downwards, the mouth becomes visible on a raised hypostome, and the overall column height is reduced. The appearance adopted resembles that of an animal anaesthetized with excess magnesium. Recovery is rapid on cessation of stimulation, an increase in column height being obvious within 2 min. The whole response might result from a loss of internal water and consequent reduction in internal pressure, but this is made unlikely by the observed rapid recovery. The shape changes may result from inhibition of the circular and parietal muscles. They will then no longer oppose the internal hydrostatic pressure and the body shape will be determined largely by the volume of enclosed water and by the structural properties and disposition of the mesogloea.

The results of SS1 stimulation are shown for comparison. The SS1 was stimulated alone by placing the stimulating electrode on a shallow ectodermal flap (McFarlane, 1969*b*). At a stimulus frequency of one shock every 10 sec at 12 °C pedal disc detachment does not occur (McFarlane, 1969*b*). Stimulation results in oral disc expansion; this is not particularly obvious here as this animal was maximally expanded at the start of stimulation. This is followed by column extension, implying that there is some method of interaction between this apparently ectodermal conduction system and the endodermal muscles. The observed response might result from an increase in internal pressure, caused, for example, by an increased rate of ciliary beating in the siphonoglyphs, but again recovery is rapid, suggesting that the internal volume has not changed during stimulation.

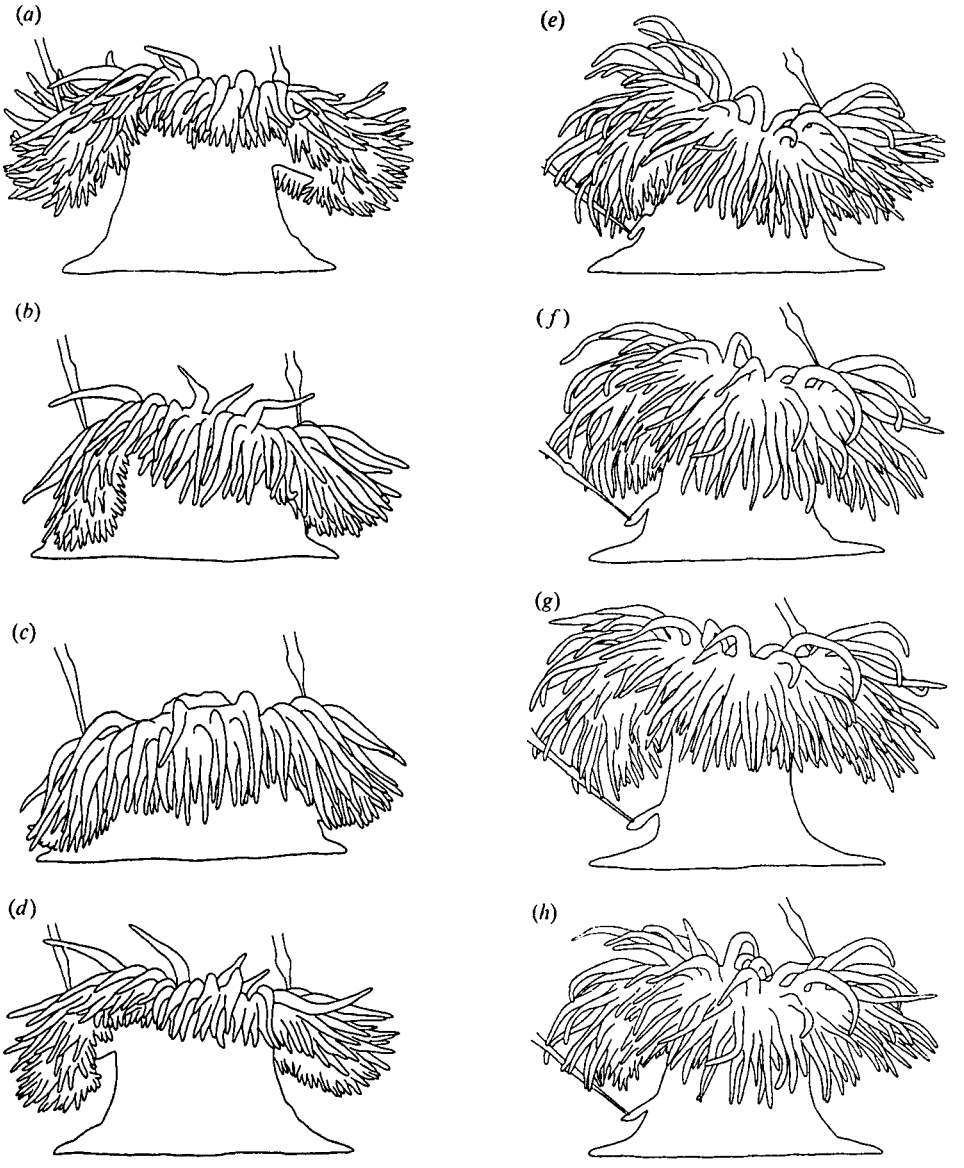

Fig. 12. Effects of low-frequency (1 shock every 10 sec) electrical stimulation of the SS2 (*a*, *b*, *c*, *d*) and SS1 (*e*, *f*, *g*, *h*) in intact *Calliactis parasitica*. Drawings made from photographs. Each sequence shows the appearance of the animal immediately before stimulation (*a*, *e*), after 2 min (*b*, *f*) and 5 min (*c*, *g*) of stimulation. Stimulation was then stopped and (*d*) and (*f*) show the appearance 2 min later.

Nerve-net stimulation at one shock every 10 sec has variable actions. A spontaneous contraction cycle is sometimes seen but this is often masked by fast contractions possibly resulting from interaction between the evoked pulses and spontaneous nerve-net pulses. Fast sphincter contraction will occur if two conducted nerve-net pulses are separated by less than 2 sec (Pantin, 1935).

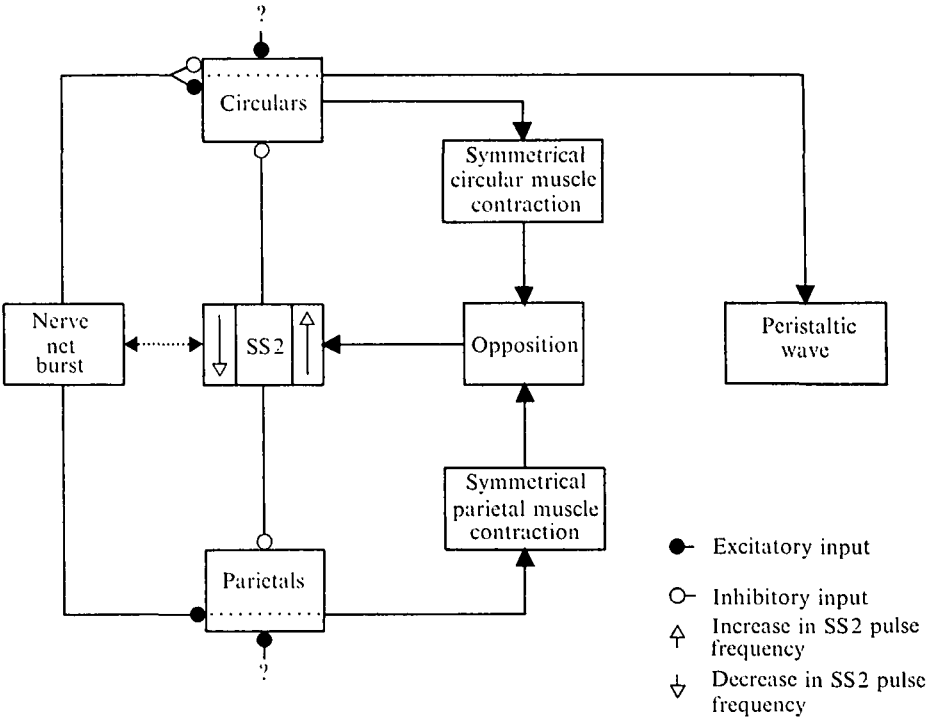

Fig. 13. Model for the control of the spontaneous contraction cycle in *Calliactis parasitica*.

DISCUSSION

Model for control of spontaneous contractions

Many spontaneously active muscle systems have been described in other animals. Regulatory control is usually provided by dual excitatory and inhibitory innervation. In *Calliactis parasitica* it seems that the parietal and circular muscles show an inherent rhythm which is modified by excitatory and inhibitory inputs from the nerve net and SS2. The conduction systems do not, however, have identical effects on both muscle groups. Some asymmetry of control is obviously necessary to co-ordinate the activities of these antagonistic muscles. The differential control of these muscles is clearly expressed in the co-ordinated sequence of contractions seen in the spontaneous contraction cycle. The mechanisms of contraction control are here discussed with reference to a simple model of the spontaneous contraction cycle (Fig. 13), each of the components of this model being discussed in turn. This model assumes that the conduction systems act directly on the muscles and not via an undetected direct or indirect link.

Nerve-net burst. Complete contraction cycles are only seen following nerve-net bursts. The frequency of pulses seen during bursts is too low to cause fast contraction of the muscles involved in the protective withdrawal response studied by Pantin (1935). Constancy of the parameters of successive bursts suggests that the pulses originate from a pacemaker (McFarlane, 1973*b*), but does not exclude the possibility that bursts result from maintained stretch-excitation of receptors. The stimulus for burst-initiation is not known but a burst occasionally follows a single nerve-net pulse elicited by mechanical or electrical stimulation. This usually happens when the evoked pulse comes some time after a spontaneous burst (McFarlane, 1973*b*). Ewer's (1960)

observation that a single shock, applied some time after a spontaneous contraction, can elicit an almost maximal contraction of circular rings may be explained on the basis of triggering of a complete burst. This then implies that the pacemaker is diffuse as it is present in completely isolated circular rings cut from different levels of the column. It does, however, seem absent from rings cut from the upper part of the column (McFarlane, 1973*a*).

Reduction in SS2 activity. Nerve-net bursts are usually preceded by a decrease in SS2 activity and accompanied by an SS2 quiet period. This reciprocal relationship between the activity of the conduction systems may be due to a direct interaction, or to an indirect link via the muscular response to the nerve-net burst, or to the activity of an undetected conduction system that affects both the nerve net and SS2. The model shows a two-way link between the nerve-net burst and the SS2 as the burst might be directly or indirectly triggered by the preceding fall in SS2 frequency. Nerve-net bursts often follow periods of SS2 stimulation (Fig. 7*a*).

The reduction in SS2 frequency and the SS2 quiet period may play an important part in the spontaneous contraction cycle by removing inhibition from the muscles and allowing them to contract.

Symmetrical parietal muscle contraction. Parietal and circular muscle preparations can show local contractions in the absence of nerve-net activity. The use of the term 'inherent' to describe these contractions implies only that the origin of their excitation is not known. Batham & Pantin (1954) proposed that inherent contractions in *Metridium senile* represent a myogenic rhythm. They dismissed the possibility of these contractions being due to stretch excitation, claiming that the amount of stretch necessary to cause contraction is far greater than that present under normal circumstances. The inherent rhythm might, however, result from the activity of a local nerve net whose conducted events do not reach recording electrodes attached to tentacles. The model simply indicates the presence of an unknown, local excitatory input responsible for the inherent activity. This activity is important as it seems to form a baseline that is raised or lowered by the nerve net and SS2.

SS2 stimulation inhibits parietal muscle preparations and cessation of stimulation is followed, after a delay of 50–60 sec, by a symmetrical contraction. This contraction seems to result not from any direct excitatory action of the SS2 but simply from a return to the inherent rhythm. A nerve-net burst may follow stimulation but is not necessary for the development of the contraction, although when it is present it may hasten the onset of the contraction and increase its size. The symmetrical parietal muscle contraction seen in the spontaneous contraction cycle may thus result not from the nerve-net burst but from a removal of inhibition of inherent activity during the period of reduced SS2 activity. Nerve-net stimulation is, however, always excitatory to parietal muscles, and the size and rate of rise of the evoked contraction are dependent on stimulus frequency. This implies that the nerve net directly excites the parietals, a suggestion supported by the observation that, over a wide range of stimulus frequencies, parietal contractions in *Metridium senile* regularly start after the 5th or 6th shock (Batham & Pantin, 1954). Histological study has shown many nerve cells in the region of the parietals in *M. senile*; these seem, however, to be sensory. These cells may connect with the through-conducting nerve net in the mesenteries (Batham, Pantin & Robson, 1960).

Batham & Pantin (1954) suggested that the nerve net may function to co-ordinate the independent inherent activity of the separate regions of the parietal musculature. They observed that parietal muscle contractions are not always precisely symmetrical, and following electrical stimulation parietal sectors may show contractions in a fixed order which is unrelated to the point of stimulation. Indeed, they proposed that the sector which contracts first in the spontaneous sequence, the leading parietal sector, may be the source of the nerve-net burst. In the present experiments it was sometimes observed that a spontaneous nerve-net burst began after the start of contraction of one strip. Perhaps the leading parietal sector is the one escaping inhibition first during the decline in SS2 frequency, and its contraction triggers the nerve-net burst that ensures more-or-less symmetrical contraction of all the parietal musculature. The presumed sensory cells connected to the through-conduction system may represent the origin of the nerve-net burst, their activity being fed into the nerve net either directly or via the postulated pacemaker system.

Symmetrical circular muscle contraction. The model shows an unknown, local, excitatory input responsible for the inherent activity of circular muscles. SS2 stimulation inhibits this inherent activity and the end of stimulation is followed, 200 sec later, by a symmetrical contraction that may result simply from a return to the inherent rhythm. The symmetrical circular muscle contraction in the spontaneous cycle comes some 100 sec after the start of the nerve-net burst. In the past it has been assumed that this contraction is directly excited by the nerve net. The long contraction latency has been explained on the basis of slow recruitment of muscle fibres (Batham & Pantin, 1954). The nerve net over the circular muscles in *Metridium senile* seems sparse and it is unlikely that all the muscle fibres are directly innervated (Batham *et al.* 1960). Nerve-net stimulation does seem to have both excitatory and inhibitory actions on the circular muscles but a period of low-frequency stimulation is followed by a local, rather than a symmetrical, contraction. The simplest interpretation of the results is that the appearance and size of the symmetrical contraction are controlled entirely by the inhibitory action of the SS2 on the inherent rhythm. The circular muscle contraction may follow the parietal muscle contraction because of the difference in the time taken to recover from SS2-mediated inhibition.

Rise in SS2 frequency. The timing of the onset of the rise in SS2 pulse frequency and the mean frequency reached seem important in controlling the extent of the contractions following a nerve-net burst. When the SS2 pulse frequency after a burst remains low, the parietals and circulars seem to relax more slowly than usual and additional contractions may appear during the interburst interval. This implies that one function of the SS2 in the spontaneous cycle may be to cut off, or damp down, spontaneous contractions. In circular and parietal muscle preparations continued SS2 stimulation during and after the nerve-net burst can almost completely prevent the development of contractions. These observations may explain why contractions appear larger in preparations than in the intact animal (Needler & Ross, 1958). It has already been noted that half-animal preparations (McFarlane, 1973*a*) differ from the preparations used here in that the SS2 activity in the former rises almost immediately after the nerve-net burst whereas in the latter there is often a considerable lag. The half-animal preparations become sealed and inflated; a functional hydrostatic skeleton is thus present. In the preparations used here the hydrostatic skeleton is open and the muscles are acting

only against a light isotonic lever, resulting in them being able to contract further without meeting opposition from the hydrostatic pressure and the action of the antagonistic muscle group. In the intact animal the damping of contractions may result purely from the mechanical effects of opposition, but the suggestion made here is that the rise in SS2 frequency is a direct result of opposition and is responsible for preventing further contraction. For example, if the circulars start contracting while the parietal muscle contraction is maintained, the muscles will be strongly opposed and this may lead to an increase in SS2 activity, causing inhibition of both muscle groups. This would function essentially as a negative feed-back system where the results of contraction are monitored and information is supplied to the muscles to modify their activity. The nerve-net burst and SS2 quiet period may release or trigger contractions; but the result is not a simple all-or-none reflex, rather a sequence of contractions that can be modified by sensory feed-back. The origin of the SS2 pulses is not known; they may arise from a sensory system detecting stress, perhaps between the muscle groups where they cross at the bases of the mesenteries, or perhaps between the muscles and the mesogloea. Alternatively, the system may respond to increased hydrostatic pressure accompanying contractions; this, however, is unlikely as the rise in SS2 frequency occurs in preparations where the hydrostatic skeleton is open. Pressure changes may, however, occur in the subepithelial fluid (Robson, 1957).

Peristaltic wave. The local circular muscle contractions that constitute the peristaltic wave are clearly related to nerve-net bursts. The delay between the end of a burst and the peak of the local contraction is about 250–300 sec for pedal rings and 400–500 sec for mid-column rings. The local contractions seen after a period of low frequency nerve-net stimulation show a delay from the end of stimulation to the peak of contraction of about 300 sec for pedal rings and 500 sec for mid-column rings. This suggests that the nerve-net burst may trigger the peristaltic wave. Batham & Pantin (1954) suggested, however, that peristaltic waves are transmitted by non-nervous spread through the muscle field, and they pointed out that such contractions are the last column contraction component to disappear during Mg^{2+} anaesthetization, continuing long after the apparent abolition of through-conducted nerve-net activity. It remains a possibility, however, that although not directly involved in the spread of the wave, the nerve net may, by virtue of its differential excitatory and inhibitory action on circular muscles, re-set the inherent rhythm and determine which region initiates the wave. It is of interest that the peristaltic wave during the spontaneous contraction cycle passes down the column in *Metridium senile* but up in *Calliactis parasitica*.

Stimulation of the SS2 after a nerve-net burst can reduce or abolish the peristaltic waves, but the frequencies involved are higher than those normally found and it is not clear whether the SS2 can modify peristaltic waves in the normal spontaneous contraction cycle.

Summary of model. The reduction in SS2 activity prior to the nerve-net burst leads to release of the parietal and circular muscles from inhibition. The inherent rhythm reappears first in the parietals, and contraction of the leading parietal sector may trigger the nerve-net burst, although alternatively the burst may result directly from the reduction in SS2 activity. The burst directly excites the parietals and ensures symmetrical contraction, the duration and pulse frequency of the burst determining the size and speed of contraction. The symmetrical circular muscle contraction that

follows is probably not directly excited by the burst and is delayed as a result of slow escape from SS2-mediated inhibition. The inhibitory action of the nerve-net burst may also contribute to the contraction delay. This contraction occurs before the parietals are fully relaxed and a stage is reached where opposition between the two muscle groups causes a rise in SS2 frequency, resulting in inhibition of contractions and perhaps an increased rate of relaxation. As the opposition is reduced the SS2 frequency falls, leading to the next cycle. The peristaltic wave may be triggered by the nerve-net burst but the mechanism of its spread is as yet unexplained.

The spontaneous contraction cycle also involves other muscles. The first visible sign of the cycle is usually twitching of scattered tentacles (Needler & Ross, 1958). This probably represents single nerve-net pulses causing fast contraction of the tentacle ectodermal muscles (Josephson, 1966). A large slow sphincter muscle contraction occurs after the symmetrical parietal muscle contraction. This is presumably directly excited by the nerve-net burst as electrical stimulation elicits slow sphincter contraction (Ross, 1957). In Ross's experiments on isolated sphincter rings, however, the contraction started early in the stimulus sequence whereas in the spontaneous cycle it is somehow delayed until after the parietal contraction.

The importance of the spontaneous contraction cycle in the behaviour of the intact animal has not been established. Needler & Ross (1958) suggested that it functioned to exchange water with the environment. The ciliary pumping action of the siphonoglyphs is presumably constant and will result in a gradual increase in internal pressure. Perhaps this pressure build-up in some way triggers the contraction cycle, leading to loss of internal water and reduction in internal pressure.

The model given here represents only a superficial view of the mechanisms involved in contraction control. Information is still required concerning the origin of the observed spontaneous pulses, the precise location of the SS2, and the mode of action of the observed inhibition. In addition, little is known about the control of local contractions such as the peristaltic wave seen in the spontaneous contraction cycle and the local parietal muscle contractions seen during other behavioural activities.

Maintenance of body shape

The feed-back system outlined in Fig. 13 could function for control of body shape, outwith the normal spontaneous contraction cycle. This would require the establishment of a reference, perhaps representing a certain mean frequency of SS2 activity. Changes in shape resulting from muscular activity or other input might result in a changed level of SS2 activity, leading to compensatory muscular contraction or relaxation. The problem then arises of what determines this level and how it might be altered to allow for establishment of a different maintained body shape such as the extended column seen during the preparatory feeding behaviour of *Metridium senile* and *Tealia felina* (Pantin, 1950; McFarlane, 1970). Basically the problem is that SS2 activity seems to inhibit both parietal and circular muscles and does not provide for reciprocal control to allow the activity of one muscle group to predominate at any one time. The only asymmetrical component in the model seems to be the inhibitory action of the nerve net on the circular muscles.

Body shape is not, however, determined solely by the action of the circular and parietal muscles; other muscle groups, the hydrostatic pressure, and the physical

properties of the body wall also play important roles. It may be assumed that the tensile modulus of the mesogloea remains constant and that changes in the mechanical properties of the mesogloea cannot initiate shape changes. Hydrostatic pressure, however, does not remain constant. It may vary on a short-term basis, rising as one muscle group contracts, thus forcing extension of the antagonist, and it may vary on a long-term basis as water accumulates in the coelenteron. If the body shape remains fixed the pumping action of the siphonoglyph will result in an increased internal pressure. The amount of activity shown by the muscles seems directly related to internal pressure. Batham & Pantin (1950*a*) showed that in *Mertidium senile* muscular contractions are most extensive when hydrostatic pressure is low. At high pressure the activity is less evident not because the muscles are quiescent but because they are contracting isometrically rather than isotonically. Maintained pressure changes may also result from feeding, the volume of ingested food increasing the internal volume, or from activity of other muscle groups; for example, relaxation of oral disc muscles could cause a fall in internal pressure.

The shape of an anaesthetized animal is presumably the basic shape imposed by the mesogloea. Under such circumstances where the muscles are inactive an increase in internal pressure will cause column extension because although the body diameter would increase twice as much as body height if the column wall was made of a mechanically isotropic material, it has been shown that in fact the tensile modulus of the mesogloea in *M. senile* is three times greater in a circumferential direction than in the longitudinal (Gosline, 1971). Alexander (1962) showed that a sample of mesogloea resists short-term stretch but increases in length slowly under continuous load; removal of the load is followed by a slow return to the initial length. Thus slow changes in muscle length and hydrostatic pressure can result in a change in body shape whereas the muscular action in the spontaneous contraction cycle will presumably involve relatively rapid stretch of the mesogloea which will then quickly return to its original length.

Comparison with muscular control in Hydra

In *Hydra* the contraction burst (CB) system seems to trigger contractions of the ectodermal longitudinal musculature causing body shortening (Passano & McCullough, 1964). Functionally the CB system may be comparable to the nerve net in sea anemones; the recorded pulse may, however, be conducted in the musculo-epithelial layer itself (Josephson & Macklin, 1967), although local nerve-net activity may function as a pacemaker (Kass-Simon, 1972). Another conduction system, the rhythmic potential (RP) system, shows rhythmic changes in spontaneous pulse frequency which are reciprocally related to changes in CB frequency (Passano & McCullouch, 1965); RP frequency falls immediately after the CB pacemaker starts firing; the RP frequency rises to its highest level immediately after a CB burst; during the period between bursts RP intervals gradually increase. The CB/RP interaction is clearly similar to the nerve net/SS2 interaction. Shibley (1969) suggests, however, that RPs trigger contraction of endodermal circular muscles. This suggests that the RP and CB system lie in different cell layers. A direct electrical link between endodermal RP events and ectodermal CB events is possible as the mesogloea between the layers is extremely thin. Alternatively, as Passano & McCullough (1965) point out, the systems might be linked by way of muscular activity.

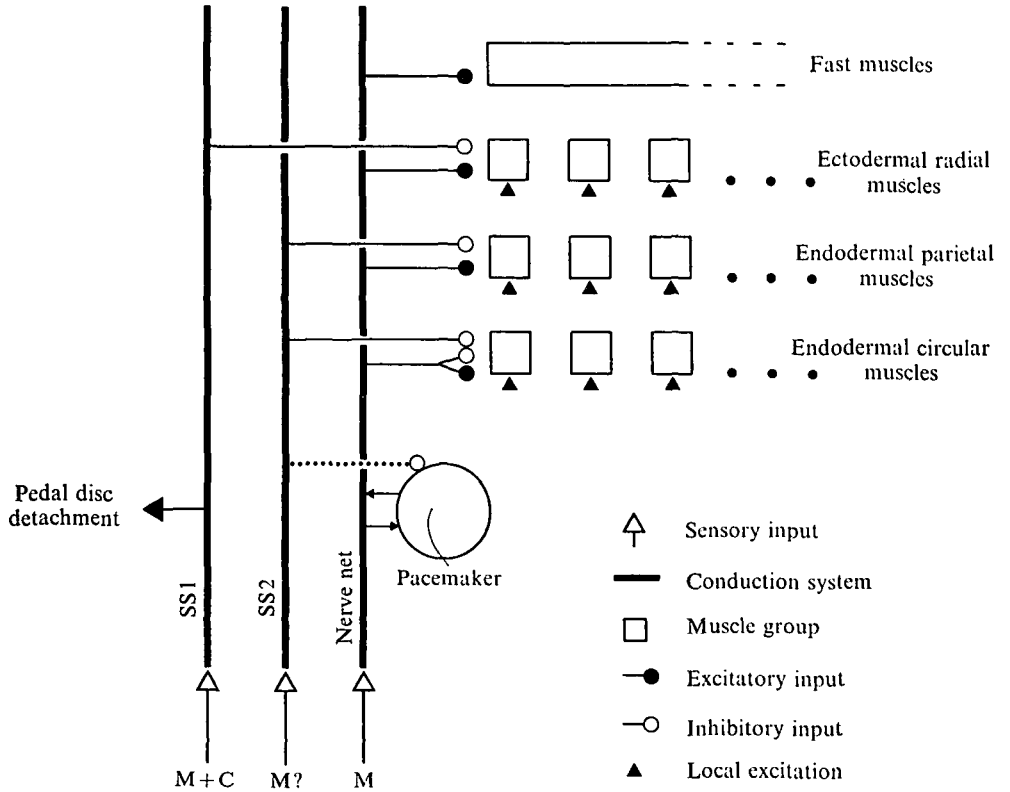

Fig. 14. Summary of the actions of the three known conduction systems in *Calliactis parasitica*. M = mechanoreceptive input; C = chemoreceptive input.

The major difference between the RP system and the SS2 is that the former triggers, whereas the latter inhibits, endodermal muscle contractions. In terms of muscle arrangement the animals differ in that the antagonists are in different tissue layers in *Hydra* whereas they are both endodermal in *Calliactis parasitica*.

Multiple conduction systems in sea anemones

The activities of the multiple conduction systems in *Calliactis parasitica* are summarized in Fig. 14. This method of presentation is based on schemes produced by Josephson & Mackie (1965) and Josephson & Uhrich (1969) in summary of studies on the hydroid *Tubularia*, but no direct comparisons between the two species are implied.

The SS1, possibly ectodermal and involving non-nervous conduction between ectodermal cells, has the unique function of co-ordinating detachment of the pedal disc during shell-climbing behaviour (McFarlane, 1969*a*,*b*). SS1 pulses can be elicited by mechanical stimulation of the column, but during shell-climbing the observed pulses may result from a specific chemosensory response associated with contact between tentacles and the *Buccinum* shell. Pedal disc detachment follows maintained SS1 activity at pulse intervals less than 10 sec. In *Tealia felina* SS1 pulses are seen following contact of dissolved food substances with the ectodermal surface of the

column (McFarlane, 1970) and the pulses lead to oral disc expansion, apparently by inhibiting inherent activity of the ectodermal radial muscles (McFarlane & Lawn, 1972). A similar response is seen in *Calliactis parasitica*, the frequency of evoked pulses being too low to cause pedal disc detachment (McFarlane, 1973*c*). It remains, however, to be directly confirmed that SS1 pulses inhibit ectodermal muscles in *C. parasitica*.

The SS2 seems to be endodermal and may also involve non-nervous conduction. SS2 pulses seem to inhibit inherent activity of endodermal circular and parietal muscles. The sensory input may be mechanical and associated with opposition between these antagonistic muscles. Both general conduction properties (McFarlane, 1973*b*) and functional properties indicate a close similarity between the SS1 and the SS2.

The through-conducting nerve net has been exhaustively analysed following the work of Pantin (1935) on the control of fast contractions. The nerve net seems to directly excite all muscles, with the possible exception described here of the circulars, and the frequency of conducted pulses determines which muscles respond and whether the muscles show fast or slow contractions (Batham & Pantin, 1954; Ross, 1957). The action of the nerve net on the column circular muscles is less certain although both excitatory and inhibitory actions may be present (Ewer, 1960). Nerve-net bursts may result from the action of a pacemaker. The reciprocal relationship between SS2 and nerve-net activity may be due to some connexion between the SS2 and this pacemaker, but the systems may be linked by way of muscular activity. The nerve net is excited by mechanical stimulation (Passano & Pantin, 1955). Some muscle groups may have a local excitatory input responsible for the observed inherent activity.

The circular and parietal muscles also contribute to other behavioural acts. Of especial interest is the remarkable shell-climbing behaviour of *C. parasitica*, a complex sequence of contractions involved in transferring pedal disc attachment to a *Buccinum* shell containing the hermit crab *Pagurus bernhardus* (Ross & Sutton, 1961). Analysis of this behaviour represents a considerable challenge, and one that can be met only by a clear understanding of the mechanisms of muscular control in sea anemones.

SUMMARY

1. Bursts of nerve-net activity are always followed by a contraction cycle involving parietal and circular muscle contractions in isolated preparations of *Calliactis parasitica*. Both muscle groups can, however, also contract in the absence of nerve-net activity. These contractions, termed inherent, seem to follow periods of reduced activity in the endodermal slow conduction system (SS2).

2. Electrical stimulation of the SS2 inhibits inherent contractions of parietal and circular muscle preparations. Electrical stimulation of the nerve net excites parietal muscles but seems to have both excitatory and inhibitory effects on circular muscles.

3. A model for control of parietal and circular muscle contractions proposes that both the nerve net and the SS2 are responsible for directing the inherent muscular activity into the observed contraction cycle. It is suggested that when the action of these antagonistic muscles is strongly opposed the SS2 pulse frequency rises, resulting in inhibition of further muscular activity.

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