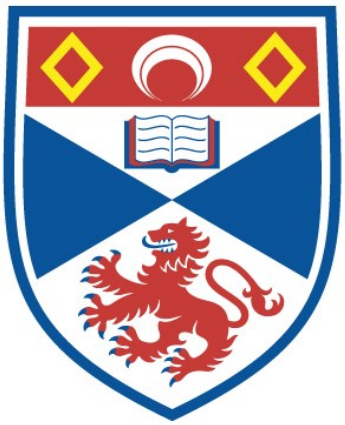
BEHAVIOURAL PHYSIOLOGY OF SEA ANEMONES

I. D. Lawn

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



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ABSTRACT

Behavioural Physiology of Sea Anemones

by

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An investigation into the problems of control and coordination of activity in the sphincter muscle of *Calliactis parasitica* has revealed the presence of a hitherto undiscovered inhibitory pathway located in the through-conducting nerve-net. The inhibitory system is responsible for delaying the onset of slow contractions in the sphincter for periods of upto 30 min or more, whereas fast contractions remain completely unaffected. It is suggested that the inhibitory effect may involve release of an inhibitory transmitter at the neuromuscular junction.

From a study of spontaneous electrical activity in unstimulated preparations it has been shown that inhibitory responses may play an important role in the control and coordination of basic behavioural activity. These investigations underline the fact that the elementary nervous system possesses a functional complexity which belies its primitive structural organization.

The nature of the mechanisms involved in the control and coordination of oral disc activity in *Tealia felina* has been investigated. It has been demonstrated that the ectodermal radial muscles of the oral disc show several different types of response. A slow conduction system, the SS1, has been shown to inhibit spontaneous activity and induce relaxation in the oral disc radials. It is suggested that these radials have a dual control system; excitatory from the nerve-net and

inhibitory from the SSl. This work emphasises the importance of the SSl as a coordinating pathway in complex behavioural activities.

Studies on sensory activity in the SSl have revealed some interesting features of sensory physiology in sea anemones. Application of dissolved food substances to the column of Tealia felina elicits activity in the SSl. This chemosensory response shows a genuine adaptation that operates over a much extended time-scale compared with sensory responses of higher animals. The response may continue for periods in excess of 1 hr.

Electrophysiological studies indicate that the chemoreceptors involved in this response are dispersed throughout the column ectoderm of Tealia, and are absent from the pedal disc and pharynx. Ultrastructural evidence is provided to suggest that these chemoreceptors are ciliated cells situated in the ectodermal epithelium of the column.

These findings are discussed in relation to the pre-feeding response of Tealia and a model for oral disc expansion has been described. The significance of the new information derived from this study is discussed in relation to the evolution both of complex behaviour patterns, and of the mechanisms involved in control and coordination of activity in sea anemones.

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A thesis presented for the degree of Doctor of Philosophy of the University of St. Andrews.

The Gatty Marine Laboratory,
The University,
St. Andrews.

July 1973.

SUPERVISOR'S CERTIFICATE

I certify that Ian Lawn has fulfilled the conditions laid down under Ordinance General No 12 of the University Court of St. Andrews, and is accordingly qualified to submit this thesis for the degree of Doctor of Philosophy.

DECLARATION

I declare that the work reported in this thesis is my own and has not previously been submitted for any other degree.

VITAE

I was educated at the Hewett School, Norwich, and the University of Nottingham where I graduated in Zoology in 1969. The work described in this thesis was carried out between October 1969 and December 1972.

ACKNOWLEDGEMENTS

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CONTENTS

INTRODUCTION

It is often assumed that the first studies on what would now be termed "The behavioural physiology of coelenterates" were simultaneously initiated by Romanes and Eimer in 1874. Over 100 years beforehand, however, The Abbé Dicquemare (1773) produced a little-known and rarely-quoted work entitled: "An Essay, towards elucidating the History of the Sea-Anemonies". In my opinion, this work should rightly be regarded as the first serious experimental study of sea anemones.

Dicquemare's main interests, as revealed in his first and subsequent essays (Dicquemare, 1775, 1777), were directed towards the developmental and behavioural physiology of sea anemones. While the second and third essays were devoted mainly to studies on regeneration and development, the first essay included a great deal of information related to behavioural physiology. A considerable variety of experiments were described, in which anemones were subjected to temperatures ranging from freezing-point upto 50°C, were placed in a partial vacuum, and suffered immersion in fresh water. Probably the most interesting study was concerned with the effects of light on these animals. Dicquemare found that although he could perceive no eyes in these creatures, they nevertheless gave a response to light. He concluded, "Is then the texture of the body of those creatures such, as not, indeed, to receive the impression of objects, with the same degree of perfection as our eyes do; but to afford a general organ affected by light, as the bodies of other animals are by feeling?" Although he found that anemones placed in the dark would close up in the presence of light and would "not spread out again 'till a while after the light was removed" he

noticed, after repeated trials, that there were exceptions to this rule: "when they had fed plentifully, they were slower in shutting themselves up, and sometimes did not close up at all". Like the earlier work by Trembley (1744) on Hydra these observations anticipated the idea that the internal state (or "Stimmung") of the animal may exert a considerable influence over its responses.

There was virtually no interest in coelenterate physiology during the 100 years subsequent to Dicquemare's studies until Romanes (1874) in England, and Eimer (1874) in Germany published their classical works on the behavioural physiology of medusae. These investigations heralded the dawn of a new era in coelenterate research and sparked off an ever gathering interest in the structure and function of the elementary nervous system.

Physiological studies during this period rested mainly on the work of Romanes (1885) on medusae and Parker (1919) on sea anemones. Several histological studies on coelenterates were also undertaken at this time, including what probably represents one of the major landmarks in the development of light-microscopy; namely the superlative work of the Hertwigs (1879-80). This work, in many respects, has rarely been surpassed, and has made a significant contribution to our understanding of the structural organization of the coelenterate nervous system.

What might be regarded as the modern era in coelenterate physiology was started by Pantin (1935) in a series of papers entitled: "The nerve-net of the Actinozoa". Our knowledge of the action of nerves and muscles in anthozoans is still largely based on the results that Pantin

obtained. The action of the nerve-net was largely deduced from indirect observations employing muscular activity as the measured output of the system. The difficulties of recording electrical activity from coelenterates are evidenced by the fact that it was not until 1953 that nervous activity was first recorded from a medusan (Horridge, 1953) and it was 13 years later that similar recordings were obtained from a sea anemone (Josephson, 1966). The major breakthrough in coelenterate electrophysiology came with the advent of suction electrodes (Josephson, 1962), and subsequent electrophysiological studies provided the first evidence that conduction systems other than the nerve-net might exist in coelenterates. It was conceivable that such conduction systems might be non-nervous, and Mackie (1965) finally established that neuroid conduction existed in coelenterates when he demonstrated propagated depolarizations in the epithelial cells covering the swimming bells of siphonophores.

Recent work on behavioural physiology of anthozoans has seen a shift in emphasis towards the study of the behavioural capacities of the intact animal and the physiological properties of its component parts, rather than regarding the whole animal simply as a neuromuscular preparation. Ross (1967b) has reviewed some of the complex behaviour patterns portrayed by sea anemones, and one gains the impression that many behavioural activities in these animals are awaiting discovery with the introduction of new methods of approach to this subject. In particular, I believe there is a special need for appropriate field-work to supplement the behavioural studies undertaken in the laboratory.

Histological studies on these animals are continually

beset by problems relating to the "difficult" nature of the tissue involved and the problems of identification of sensory and nervous elements. Despite this, the work by Pantin's school of researchers, Batham and Robson notable amongst them, has considerably furthered our knowledge of the structure of the nerve-net in anthozoans. Unfortunately, these animals have so far proven rather unsuitable for studies with the electron microscope, and it is to be hoped that suitable ultrastructural techniques will be developed in the near future to assist our understanding of the relationship between structure and function in this group.

The electrophysiological approach to the study of sea anemones was first utilized by Josephson (1966) using the tropical sea anemone Calliactis polypus. One can judge the quality and depth of perception expressed in Pantin's original work by the fact that Josephson's study essentially confirmed Pantin's earlier observations. At about this time it was becoming increasingly evident that the complex behaviour sequences encountered in many sea anemones were seemingly beyond the organizing capabilities of a single nerve-net. It was hoped that electrophysiological recordings from anthozoans would reveal the presence of multiple conduction systems in order that the control and coordination of complex behaviour might be adequately explained. McFarlane's (1969b) important discovery of two slow conduction systems (the SS1 and the SS2) in the sea anemone Calliactis parasitica has opened up a completely new approach to the behavioural physiology of anthozoans. Previous to this all aspects of control and coordination in sea anemones were assigned to the nerve-net alone. The demonstration of multiple conduction systems has

called for a re-examination of many aspects of behavioural physiology in these animals, and the work presented in this thesis is directed towards that end.

The SS1 features predominantly in these investigations, and therefore the properties and possible nature of this conduction system will be outlined here. It has been tentatively suggested that the SS1 is an epithelial conduction system located in the ectoderm of sea anemones (McFarlane, 1969b). Unfortunately, unlike Mackie's (1965) demonstration of epithelial conduction in siphonophores, there is no unequivocal histological evidence to verify this. The following factors, however, all tend to suggest that the SS1 may be located in the ectodermal epithelial cells.

a) Pulses (SPIs) associated with the SS1 may be evoked by low-intensity stimulation of ectodermal surfaces only (McFarlane, 1969b).

b) Stimulation of a superficial flap cut in the ectoderm of the column excites the SS1 only (McFarlane, 1969b), whereas stimulation of the mesogloea directly under the flap excites the through-conduction system and the endodermal slow system (SS2). Such flaps consist of ectoderm and superficial mesogloea, and thus there is a remote possibility that the SS1 may reside in the superficial mesogloea. The mesogloea in actinians, however, is not an epithelium but may be described as a two-phase system of collagen fibres embedded in a matrix (Gosline, 1971). The idea that nerve fibres may be present in the mesogloea has been challenged by Batham, Pantin & Robson (1961). They carried out an extensive structural study of the mesogloea in Metridium senile, using both light and electron microscopy, and confirmed the essential separateness

of the epithelia from the underlying mesogloea. They also noted the general absence of cells in the mesogloea, other than amoebocytes. Occasionally, genital cells are also found within the mesogloea (Pantin, 1960), but Batham et al. were unable to demonstrate the presence of mesogloeal nerve-cells and concluded: "It is hard to prove a negative. But the results of many years' work by us seem unequivocally to support the position of the Hertwigs..... that there are no mesogloeal nerve-fibres in actinian tissues we have investigated".

Until convincing evidence to the contrary appears, one can, perhaps, assume that conducting elements are absent in the columnar mesogloea of actinians, and that the SS1 is truly located in the ectoderm.

c) The evidence for and against the existence of a nerve-net in the ectoderm of the column is outlined in Part Two. It is concluded that the histological evidence does not allow one to determine whether the SS1 is a nervous or non-nervous system on the basis that it is difficult to prove the absence of nerve elements. Non-nervous conduction, however, has been demonstrated in siphonophores (Mackie, 1965) and hydromedusae (Mackie & Passano, 1968), and it is clearly possible that a similar, non-nervous conduction system could exist in actinians too.

d) The SS1 is susceptible to magnesium anaesthetization (McFarlane, 1969b), which could suggest a nervous basis; but the extremely low conduction velocity (4-12 cm/sec) the long refractory period (relative refractory period about 1400 msec, absolute refractory period about 300 msec) and the lability of the SS1, tend to suggest that the system is non-nervous.

Although the properties of the SS1 contrast strongly with those of the through-conduction system they do not, however, deny a nervous basis for the SS1.

e) Activity in the SS1 can be recorded from other regions of the ectoderm besides the tentacles. The possibility that the SS1 is a muscle action potential can be eliminated, as there is no ectodermal musculature in the column. In addition, the large SP1s recorded from the sphincter region (McFarlane, 1969b) cannot represent a slow contraction of the sphincter muscle, as both fast and slow contractions are elicited at the threshold of the through-conduction system (Ross, 1957), which is below that of the SS1.

f) The smooth biphasic or triphasic shape and the relatively long duration (often exceeding 50 msec) of the SP1s contrast sharply with the shape and duration of those pulses believed to be recorded from the neurites of the through-conduction system (McFarlane, 1969b). One would expect that a pulse with a variable duration and complex waveform, like that of the SP1, would be recorded from a multitude of excitable cells grouped under the recording electrode. Such a waveform could result from activity in a number of parallel neurites, but no such structures have ever been described in the column ectoderm. It would be simpler to believe that the observed SP1s result from activity in the epithelial cells themselves. On the basis of the evidence outlined above, it is perhaps fair to assume that the SS1 is a slow conduction system located in the epithelial cells of the ectoderm.

PART ONE of this thesis deals with the problems of control and coordination of activity in the sphincter muscle of Calliactis parasitica. The importance of this work lies in

the fact that an inhibitory pathway has been discovered in the through-conducting nerve-net, and emphasises the need for continued investigation into the properties of the so-called elementary nervous system. PART TWO examines control and coordination in the oral disc of Tealia felina, and underlines the importance of the SSl as a coordinating pathway in complex behavioural activities. PART THREE is concerned with sensory input to the SSl and has brought to light some interesting information on sensory physiology in sea anemones.

Finally, the GENERAL DISCUSSION considers the possible significance of this new information in explaining the means by which complex behaviour patterns and mechanisms of control and coordination may have evolved in sea anemones.

PART ONE

EXCITATION AND INHIBITION IN THE MARGINAL SPHINCTER OF CALLIACTIS PARASITICA.

INTRODUCTION

Much interest in actinian neuromuscular physiology has stemmed from Pantin's (1935a) classical analysis of the quick facilitated response in the marginal sphincter of *Calliactis parasitica*. His investigations helped to reveal the nature of the quick response and showed that a single pulse in the nerve-net does not normally evoke a contraction but facilitates the response in such a way that subsequent pulses, arriving before the decay of facilitation, will elicit fast contractions. Further studies revealed that stimulation of *Calliactis* at frequencies too low to evoke the quick response succeeded in exciting other, slower muscle systems within the animal (Pantin, 1935b).

Slow responses were studied in more detail by Batham and Pantin (1954) who found that isolated sphincters and mesenteric retractors of *Metridium senile* were capable of both fast and slow contractions. Ross (1957) demonstrated the presence of a slow response in isolated sphincter preparations of *Calliactis* in addition to the familiar quick, facilitated response. He proposed that the same muscular machinery was employed in both cases and ventured the possibility that each was served by different motor pathways. From observations of whole animals and isolated preparations of *Calliactis*, Needler & Ross (1958) concluded that in those actinian muscles capable of both fast and slow contractions

the quick response appeared to be a specialized mechanism for rapid closure and was superimposed upon, and perhaps developed from, a more primitive and general slow contractile mechanism. They report that spontaneous, fast contractions were rarely seen in unstimulated, intact Calliactis, although one must bear in mind that these particular anemones were removed from their shells and were therefore not subject to the buffeting that would normally be encountered whilst being carried by commensal hermit crabs. The results of their studies led Needler and Ross to propose that, as far as the general behaviour of Calliactis was concerned, the majority of neuromuscular activity, even in the marginal sphincter itself, consisted of slow contractions. This would suggest that the classical ideas of actinian neuromuscular physiology were based on studies of a system displaying relatively specialized activity.

The majority of physiological studies on cnidarians have been concerned with excitatory systems, whereas comparatively little is known of inhibitory mechanisms. Recently, however, there has been a mounting interest in this field and its significance is gradually being realised. Several examples of inhibitory phenomena in cnidarians have been tabulated by Josephson & Uhrich (1969) and an examination of the literature reveals that although inhibition has been observed in several groups in no case is the inhibitory mechanism understood.

Batham & Pantin (1954) reported reciprocal inhibition between parietal and circular muscles in the column of Metridium senile. Horridge (1955) demonstrated that contraction of radial muscles in the manubrium inhibits rhythmical

contractions of circular muscles in the bell of Aequorea forskalea and several other hydromedusae. An almost identical effect was found to occur in a number of scyphomedusae (Horridge, 1956b). Continuous electrical stimulation of the alcyonarian Heteroxenia fuscescens seems to partially inhibit tentacular beating in all the zooids of the colony (Horridge, 1956a).

Hoyle (1960) suggested the possibility of reciprocal inhibition between diametrically opposed parieto-basilar muscles during the swimming response of the sea anemone Stomphia coccinea. He suggested that a mechanism existed whereby the "tonic" nerve discharge to the "contralateral" parieto-basilars is inhibited when the "ipsilateral" ones are strongly excited. These suggestions were, however, based upon indirect evidence. Robson (1971) reports that high-frequency electrical stimulation inhibits tentacle strokes in Gonactinia prolifera, and this may be closely related to Davenport's (1962) findings that similar stimulation inhibits spontaneous activity in isolated tentacles of Radianthus. In Stomphia Ross & Sutton (1964b) have shown that previous contact with food substances can inhibit the swimming response normally elicited by contact with Dermasterias (starfish).

The first demonstration of inhibition in Calliactis was presented by Ewer (1960) who found that continuous low-frequency stimulation inhibited spontaneous activity of isolated circular muscles taken from the pedal disc and column. Behavioural studies on the association between hermit crabs and the tropical anemones Calliactis polypus and C. tricolor (Ross & Sutton, 1968, 1970) indicated that the crab's behaviour tended to produce a state of inhibition throughout the anemone,

causing it to open and become completely relaxed, toneless and unresponsive as long as stimulation was maintained. This presumably aided the transfer from substrate to crab.

Since the advent of electrophysiological recordings from cnidarians (Horridge, 1953) it has proven possible to relate some of the observed inhibitory responsesto activity in the conduction systems. These studies have been devoted almost exclusively to hydrozoans. It was found that endogenous contraction bursts in Hydra could be inhibited by localized illumination of the sub-hypostomal region or the lower stalk of the animal (Passano & McCullough, 1962, 1963). Single electrical shocks applied to the column were found to produce a similar effect (Passano & McCullough, 1964). Josephson & Uhrich (1969) have indicated that stimulation of the distal opener system (DOS) in the hydroid Tubularia inhibits pacemaker activity. It seems to suppress both single pulses and bursts in the neck pulse (NP) pacemaker system and may also inhibit hydranth pulse (HP) pacemakers. One important outcome of their findings was that the NP pacemaker system could be modulated by distinct excitatory (triggering system-TS) and inhibitory (DOS) inputs.

More recently, studies of this type have been commenced with anthozoans. McFarlane & Lawn (1972) have demonstrated that activity in the SS1 of Tealia felina inhibits spontaneous activity and induces relaxation of the radial muscles in the oral disc (see Part Two). McFarlane (1973a) has noted that an increase in spontaneous SS1 activity in Calliactis parasitica is often accompanied by a corresponding decrease in nerve-net and SS2 activity. He has suggested that this may represent pacemaker inhibition.

The work presented here is the first clear demonstration in anthozoans of an inhibitory response mediated by the through-conduction system and not involving a reduction of spontaneous activity. It is of further interest in that active inhibition is demonstrated in the marginal sphincter of Calliactis, for it is this muscle on which the basis of actinian neuromuscular physiology has been founded and to date has been assumed to show excitatory responses alone.

MATERIALS AND METHODS

Specimens of Calliactis parasitica were obtained from the Marine Laboratory, Plymouth and maintained in the aquarium at the Gatty Marine Laboratory for at least two months. This permitted them to adjust to the different water conditions and to recover from damage sustained during transit. The anemones seemed to thrive in the water temperatures encountered at St. Andrews (4-14^{o}C) and readily attached themselves to Buccinum shells. Only healthy specimens with expanded oral disc diameters of 4-6 cm were employed. These were starved for three days before use to ensure that the results of the experiments would not be modified by phasic activity associated with the digestive cycle (Batham & Pantin, 1950b).

The preparations used in the initial experiments were obtained in the following manner. An anemone was bisected longitudinally and a thread sewn into the thickest region of the sphincter muscle, about 5 mm from one of its cut edges. The sphincter of Calliactis is a large muscle occupying a zone, at the top of the column, upto 1 cm deep in an anemone with a column 3 cm in diameter (Robson, 1965). The muscle fibres themselves are arranged in groups within the mesogloea.

It was found that the tissue in this region was quite firm and tearing did not present a problem. The half-animal was then removed from its Buccinum shell and pinned along the other cut edge of the column. The sphincter region of this edge was securely pinned down so that any contraction of the sphincter muscle would be registered directly through the thread attached to its free edge. The thread was, in turn, attached to a light isotonic lever writing on a kymograph.

Later experiments employed preparations in which a strip of sphincter muscle was partially isolated from the other muscle groups. This merely involved further dissection of the half-animal preparation. An incision was made in a direction parallel to the sphincter muscle to separate its thickest region from the restbof the column. The incision extended from the free edge of the preparation to within 1cm of its fixed edge. This leaves a narrow bridge of intact column which provides a pathway for the three conduction systems (nerve-net, SS1, SS2) thus enabling propagated electrical activity to reach all parts of the preparation. The mesenteries attached to the sphincter strip were excised, whereas those below it were left intact in order to preserve a through-conducting pathway for the nerve-net. Most of the oral disc region was trimmed away from the strip, although a few tentacles were left attached at its base so that electrical activity could be recorded during stimulation.

It was found that removal of the pedal disc prevented it from curling over and sealing the exposed coelenteron. If this sealing process were allowed to occur a partial restoration of the hydrostatic skeleton would ensue, and by experience this was found to accentuate the movements of the

column musculature, thereby interfering with recordings of sphincter activity. In addition, the preparation was set up in such a way that the attached thread pulled the strip slightly away from the rest of the column. This ensured that the strip was never in contact with movements occurring in the column, and in practice this was found to eliminate the majority of extraneous movement artifacts on the kymograph recordings.

A shallow flap, to which a stimulating electrode may be attached, was cut near the base of the column. Such a flap, comprises ectoderm and superficial mesogloea, and this technique allows stimulation of the following systems (McFarlane, 1969b):

1) The nerve-net alone - by electrical stimulation of either the mesogloeal surface under the flap or of a mesentery. The applied voltage should be only just above threshold to avoid stimulation of the SS2. The nerve-net may also be stimulated with the electrode on the intact column and the stimulus voltage below SS1 threshold. The SS1 threshold is about 50% higher than that of the nerve-net.

2) The SS1 alone - by electrical stimulation of the flap at voltages above SS1 threshold.

3) The SS1 and nerve-net together - by stimulation of the intact column at voltages just above SS1 threshold.

All operations were performed without anaesthetic as the preparations seemed to recover more quickly and were generally more active than when anaesthetics were used. Preparations were left to recover in running, well-oxygenated sea water for 6-48 hr, depending on the age required. During experiments they were retained under similar conditions at temperatures between 7-12°C.

Suction electrodes were used for both recording and

stimulating (Josephson, 1966). These were constructed from polythene tubing drawn out over a low flame to give a relatively short taper to the electrode tip (internal diameter about 0.5mm). The tip was made relatively wide in order to keep the electrode resistance as low as possible. A length of chlorided silver wire was inserted to within 5 mm of the tip of the suction electrode (total length about 5-10 cm) and this assemblage was attached to a 1 ml hypodermic syringe. The joint was sealed with dental wax from which the silver wire was allowed to project. This was soldered to a low-noise, screened miniature cable leading to one channel of the pre-amplifier in the recording circuit. The plunger of the 1 ml syringe was removed and a length of flexible polythene tubing inserted. This was then connected to a 10 ml syringe so that sea water could be drawn into the system until contact was made with the silver wire. The tip of the electrode could then be placed against the surface of the animal and suction gradually applied so that contact could be maintained. Too much suction might easily damage the tissue, especially when recording from the delicate tentacles and oral disc, whereas too little suction would allow the animal to throw off the electrode relatively easily. By a process of trial and error it is possible to apply the correct amount of suction to provide perfect recording conditions. An indifferent electrode, consisting of chlorided silver wire, was usually wound around the outside of the suction electrode and held in place by the dental wax. This wire was also soldered to low-noise cable and connected to the other channel of the pre-amplifier.

Signals were fed to conventional capacitor-coupled differential pre-amplifiers (Tektronix 122). These were modified

slightly to give a bandwidth between 8 and 30 Hz, as this proved the best frequency response range for recording electrical activity from sea anemones. This is because the recorded pulses are of relatively long duration, and hence a high-frequency response is unnecessary. By keeping the upper limit of the response range below 50 Hz some hum may be eliminated without greatly affecting the size of the recorded pulses. The amplified pulses were displayed on a Tektronix 564B storage oscilloscope, and stored sweeps were photographed with an oscilloscope camera.

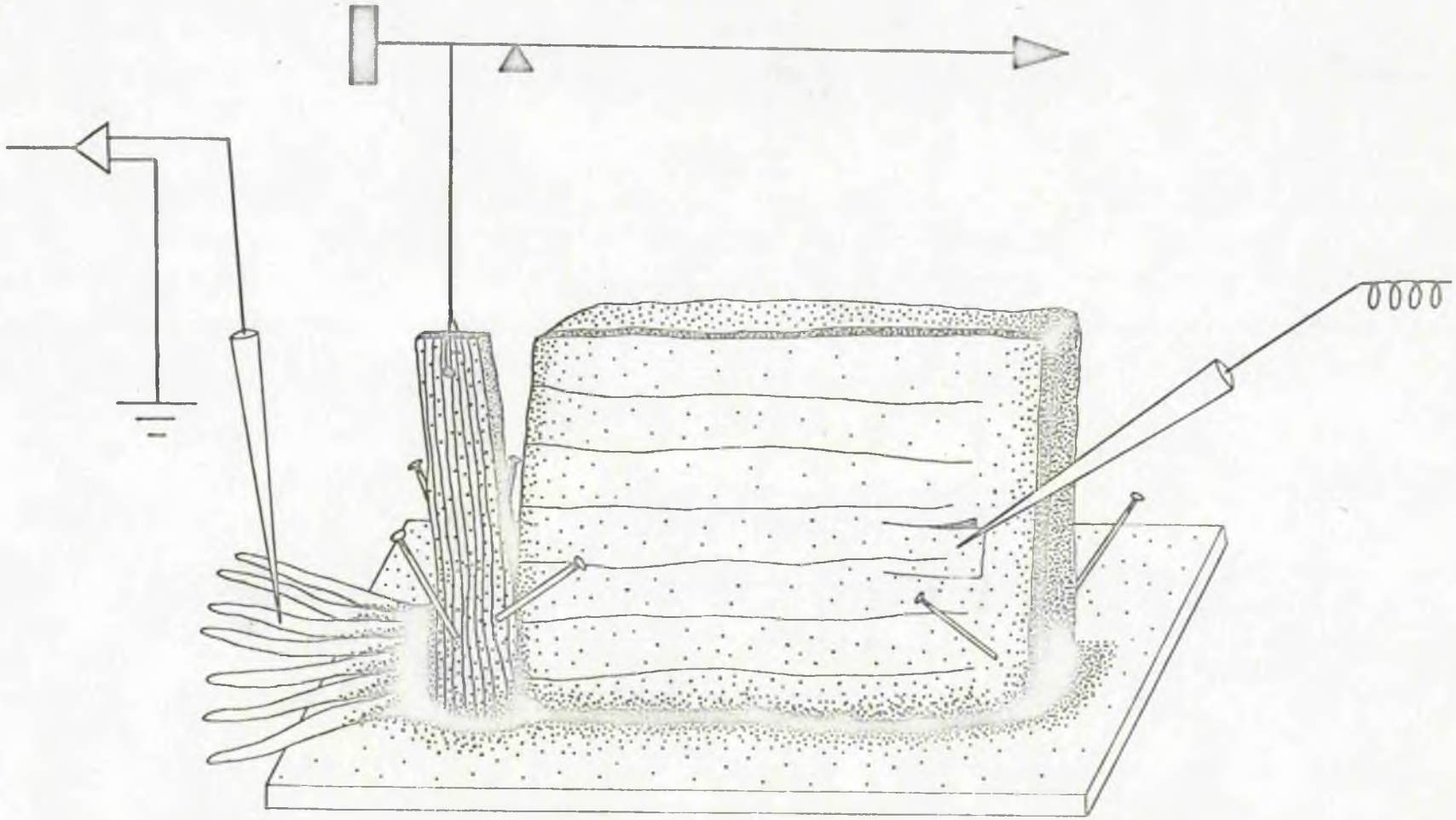
Suction electrodes were also used for electrical stimulation. A Devices Digitimer and Devices Isolated Stimulator proved the best stimulation system for this type of work, as the noise level was found to be very low. This system was employed to deliver single stimuli and stimulus trains at high and low frequencies. All electrical stimuli were of 1 msec duration.

The experimental arrangement for the sphincter strip preparation is summarized in Fig. 1.1. The recording electrode was always attached to the mid-region of a tentacle and was used to monitor the results of electrical stimulation. This is essential with the flap-stimulation technique in order to ensure that the desired conduction system is being stimulated.

It is perhaps worth mentioning that one often finds it difficult to obtain consistent results from different preparations. If great care is taken, however, it is possible to make some generalizations concerning their behaviour, although several experiments with many different preparations must be observed before one can determine whether certain properties are genuine responses, or merely individual variations between

Fig. 1.1. The sphincter strip preparation. Suction electrodes were used for recording and stimulation. The recording electrode is attached to the mid-region of a tentacle. The stimulating electrode is here shown attached to a flap in order to stimulate the SSl. Details of other stimulus electrode positions are given in the text. Note the narrow bridge of intact column below the sphincter strip. This provides a pathway for through-conducted activity to reach all parts of the preparation.

1.1



animals. All the results presented in this study are believed to be genuine responses found in the majority of animals and preparations.

RESULTS

Pulse Types

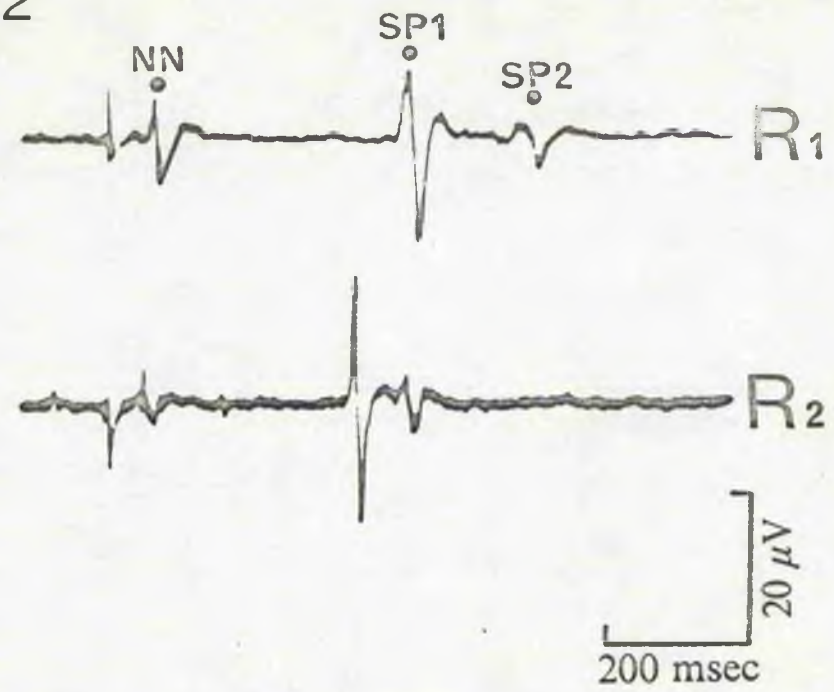
Suction electrodes record three pulsetypes associated with propagated electrical activity in Calliactis parasitica (McFarlane, 1969b, 1973a). These are associated with the through-conduction system of the nerve-net and two slow systems (SS1 and SS2). These pulses are often very small and it was found that they could be identified more readily by comparing activity from two recording electrodes (R_1, R_2) attached to tentacles 1-3 cm apart. Fig. 1.2 shows recordings of activity evoked by electrical stimulation of the intact column at an intensity above the threshold of the SS2. These recordings indicate that the site of stimulation is nearer to R_2 than R_1 as the propagated pulses arrive earlier at R_2 and hence travel a shorter conduction pathway from the stimulating electrode.

The pulse associated with the through-conducting nerve-net (NN) is rapidly conducted (up to 120 cm/sec) and is evoked by a single shock to any part of the anemone (McFarlane, 1969b). It often appears as an all-or-none pulse of short duration (less than 10 msec) with an amplitude ranging from 4-15μV. Similar responses have been recorded from the mesenteries of Calamactis praelongus (Pickens, 1969) and Metridium senile (Robson & Josephson, 1969) and from tentacles of Tealia felina (McFarlane, 1970). These pulses possibly represent activity in the neurites of the through-conducting

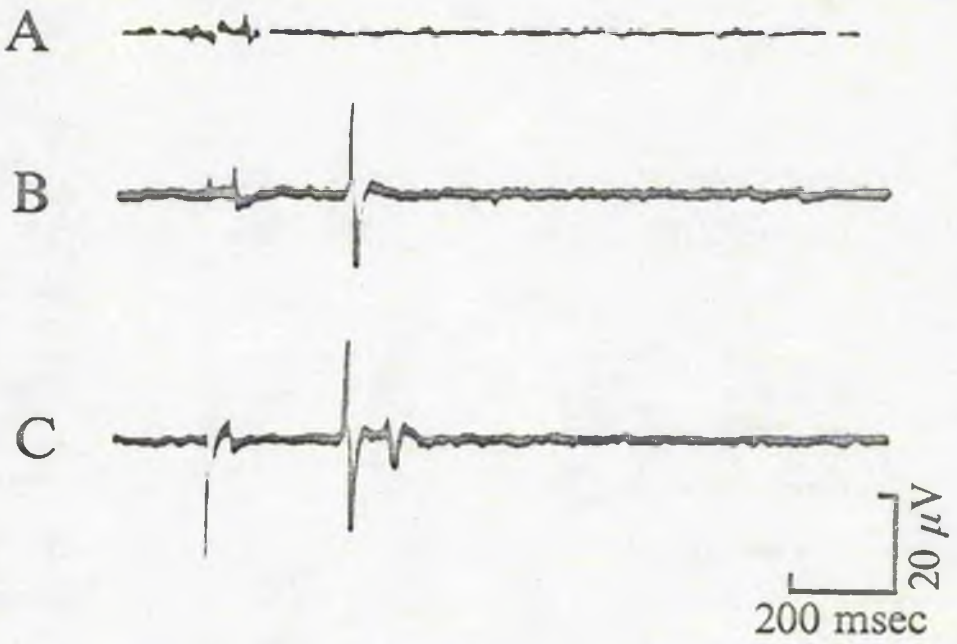
Fig. 1.2. Pulse types encountered in Calliactis parasitica. The two recording electrodes (R1 and R2) are attached to tentacles 3 cm apart. Recordings indicate that a single electrical shock applied to the intact column at a stimulus intensity above the threshold of the SS2 elicits propagated electrical activity associated with three conduction systems. The pulse associated with the through-conducting nerve-net (NN) is rapidly conducted and appears almost simultaneously at Rland R2. Both the SS1 pulse (SP1) and the SS2 pulse (SP2) are slowly conducted and hence show a considerable difference in time of arrival at the two recording sites.

Fig. 1.3. Stimulation thresholds in the three conduction systems. Recordings taken from a single recording electrode attached to a tentacle. Single electrical shocks of 1msec duration were applied at different intensities through a stimulating electrode applied to the intact column. Stimulus intensities: (A)2V, (B)7V, (C)25V. A pulse in the through-conducting nerve-net is the only electrical activity recorded in A. As the stimulus intensity is gradually increased (B) the threshold of the SS1, and finally (C) the threshold of the SS2 is surpassed. These results indicate that the through-conducting nerve-net and the SS2 have the lowest and highest stimulus thresholds respectively, under these conditions of stimulation.

1.2



1.3



nerve-net, for in Calliactis a second shook, closely following the first, produces a large muscle action potential and an associated fast contraction of the tentacles and sphincter (Josephson, 1966; McFarlane, 1969b).

The nerve-net pulses in Calliactis may vary slightly in shape and size (McFarlane, 1973a) and one sometimes encounters more complex pulses of longer duration which may represent either summated activity in a number of neurites, or a small muscle action potential accompanying a small twitch of the tentacles (Josephson, 1966). In a given recording position, however, the shape and duration of the pulse remains reasonably constant and is easily distinguished from slow-system pulses. With some attachments the nerve-net pulses were not clear, and in these cases the electrode was moved to another site until a good recording position could be obtained. This suggests that these pulses are not muscle action potentials, associated with a small contraction of the tentacle longitudinals, but activity recorded from the scattered neurites themselves (McFarlane & Lawn, 1972).

The SS1 pulse (SP1) is the large biphasic event following the nerve-net pulse. SP1s are typically biphasic or triphasic pulses with an amplitude sometimes exceeding 30μV in Calliactis (McFarlane, 1969b), and a duration varying between 50-200 msec. Because of the low conduction velocity (3-14 cm/sec) the pulses in the SS1, like those in the SS2, often show a considerable difference in time of arrival at the two recording sites. The SS1 is thought to be ectodermal (McFarlane, 1969b) and a single pulse spreads over the entire ectoderm in response to a single shock to any ectodermal region. The properties and actions of the SS1 suggest that

it is a non-nervous conduction system (see Introduction to this thesis).

The pulse associated with the SS2 (SP2) is usually smaller than the SP1 and rarely exceeds 10μV (McFarlane, 1969b, 1973a). Its duration is variable, like that of the SP1, and conduction velocity is low (about 4 cm/sec). The shape of the SP2 shows considerable variation but is often biphasic and predominantly positive. Although the nerve-net pulses and SP1s are always clear and easy to recognise the SP2s are sometimes not so obvious and their identification is facilitated by comparing pulses in the two recording electrodes. Both slow systems show an increased response delay with repeated stimulation. The SS1 shows evidence of fatigue even when stimuli are 20 sec apart (McFarlane, 1969b) and conduction in the SS2 is even more likely to fail under these conditions. The refractory period of the SS2 is difficult to determine but appears to be approximately 1 sec (McFarlane, 1973a). SP2s have been recorded only from the tentacles and it is thought that the system may be endodermal (McFarlane, 1969b).

The three conduction systems all have different thresholds of stimulation. Fig. 1.3 shows the activity monitored in a single recording electrode in response to single stimuli administered by a stimulating electrode attached to the ectodermal surface of the intact column. In Fig. 1.3A a stimulus intensity of 2V was applied, and this evoked a single impulse in the through-conducting nerve-net. Fig. 1.3B shows the response obtained at an intensity of 7V, and shows that a pulse in the SS1 is evoked in addition to that in the nerve-net. At an intensity of 25V pulses associated with the nerve-net, SS1 and SS2 are recorded (Fig. 1.3C). All electrical

stimuli in the following experiments were administered to the ectodermal surface of the lower column at intensities above the threshold of the SS2, except where stated otherwise.

The great range of variation in the size and shape of pulses encountered in recordings from sea anemones is probably due to a combination of the recording techniques employed and the nature of the tissue from which recordings are being made. The means by which electrical activity is detected by suction electrodes is not fully understood, although presumably a good insulation between the inside of the recording electrode and the surrounding bath is essential in order to ensure a maximum difference between the recorded signal and the ground potential of the bath. The nature of the seal formed between the tip of the suction electrode and the tissue of the animal is, therefore, probably a critical factor in this type of extracellular recording. This may explain why it is possible to record larger and clearer pulses consistently from some animals rather than others. In specimens producing good recorded pulses it is possible that their surface layers are flaccid and hence capable of forming a more efficient seal with the suction electrode. This might also explain why it is possible to get far better recordings from tentacles than any other region of the anemone. The longer a recording electrode is left on the animal the better the seal should become, and this should produce clearer pulses. This is consistent with the observation that pulses generally increase in size over the first 30 min or so following electrode attachment, and then remain at a fairly constant size. It was noted that occasionally, if the electrodes were left attached for several hours, the pulse sizes would again decrease,

possibly owing to damage of the tissue under the electrode whilst under suction. For this reason, whilst monitoring for long periods, just enough suction was applied to prevent the anemone from throwing off the electrodes. This was found to be gentle enough to avoid tissue damage for many hours, and hence long term recordings could be accomplished without need to renew the electrode position.

Responses to Electrical Stimulation

Stimulation of the isolated sphincter of *Calliactis parasitica* seems to evoke fast or slow contractions, depending on the frequency of stimulation (Ross, 1957). The situation in the intact animal may be different, however, for in *C. polypus* low-frequency stimulation not only fails to elicit slow contractions but seems to induce a state of general inhibition throughout the animal (Ross & Sutton, 1968). The aim of this study is to examine the effects of low-frequency stimulation on the sphincter of *C. parasitica* in order to establish whether the inhibitory effects suggested from observations of the intact animal can be reproduced in sphincter preparations. I decided to look at the problem by initially studying the inhibitory effect in the intact animal and then attempted to reproduce it in various preparations.

1) *Intact Animals*: The tropical anemones *Calliactis polypus* and *C. tricolor* respond to low-frequency mechanical stimulation of the column by opening and relaxing (Ross & Sutton, 1968, 1970). This mimics the behaviour of the pagurids living in commensal association with them, for the crabs seem to induce a general inhibition by palpating the column of the

anemones during the detachment response. This induced state of relaxation presumably facilitates transfer by preventing the anemone from contracting during manipulation by the crab.

The European species, C. parasitica, also enters into a symbiotic relationship with pagurids; Dardanus arrosor in the Mediterranean and Pagurus bernhardus in the English Channel. D. arrosor seems to play an active role in establishing the association. It palpates the column and induces a state of relaxation (Faurot, 1910; Brunelli, 1910, 1913), although Ross & Sutton (1961b) point out that the primary factor involved in this partnership is the response of Calliactis to a molluscan shell factor. The shell response seems to be the only factor concerned in the association of C. parasitica with P. bernhardus, for the crab seems to play no part in assisting the transfer of the anemone (Ross, 1960c; Ross & Sutton, 1961a).

My own observations on C. parasitica from Plymouth indicate that a general inhibition can be induced by stroking the column gently with a blunt instrument. At frequencies above 1 stroke every 2 sec the anemone usually contacts strongly and may remain so until stimulation ceases. This is the familiar protective withdrawal response, involving a quick facilitated contraction of the marginal sphincter (Pantin, 1935a). Stroking at frequencies below 1 every 3 sec, however, seems to relax the anemone completely. It may retract to the first few strokes but the inhibitory state seems to develop as stimulation proceeds, and eventually the anemone may be treated quite roughly before retraction occurs. These responses to mechanical stimulation seem identical to those described by Ross & Sutton (1970) for C. polypus and C. tricolor, the

only apparent difference being that the quick facilitated response in the tropical anemones occurs at higher frequencies of stimulation, due to the higher environmental temperatures (26-30°C).

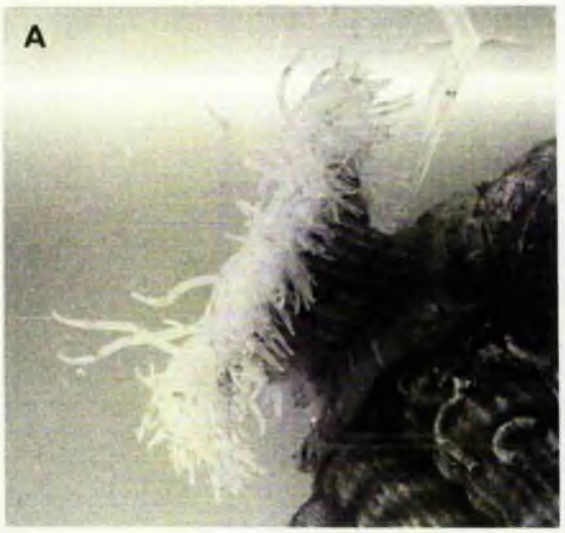
These preliminary observations indicated that the basic motor mechanism that produces general inhibition (Ross & Sutton, 1968) is also present in C. parasitica, and hence this species would prove suitable for an investigation into the phenomenon of sphincter inhibition.

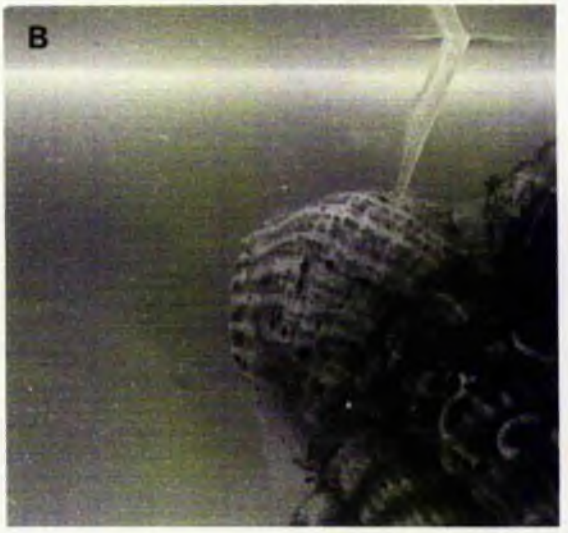
Electrical stimulation of intact C. parasitica at frequencies below 1 shock every 2 sec elicits inhibitory effects identical to those observed with mechanical stimulation. The sequence in Fig. 1.4 shows this response to electrical stimulation at a frequency of 1 every 5 sec. In Fig. 1.4A the anemone is shown at rest before stimulation commences. When the first 3-4 shocks of the series are applied the anemone often retracts (Fig. 1.4B) but eventually opens and remains relaxed throughout the period of stimulation (Fig. 1.4C and D). Shortly after the cessation of stimulation, however, the sphincter seems to contract slowly, as seen in Fig. 1.4E, before returning to the resting state (Fig. 1.4F). During stimulation some slow movements of the column were noted, and a slow depression of the oral disc often occurred, an effect also noted in C. polypus (Ross & Sutton, 1970). The depression of the oral disc is probably due to slow contraction of the longitudinal mesenteric muscles, as these are excited at frequencies below those which cause sphincter contraction (Pantin, 1935b).

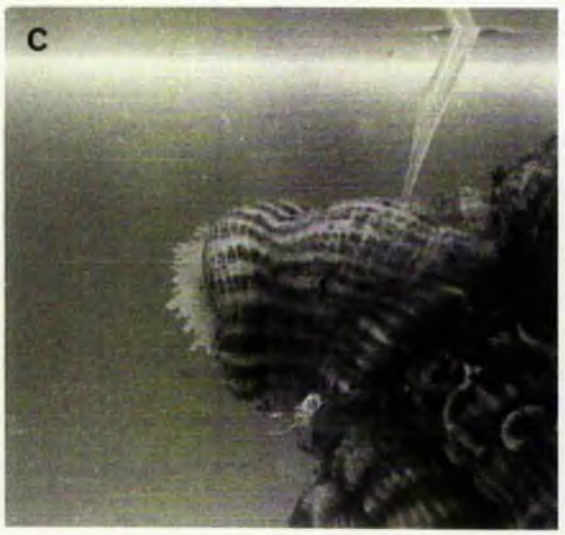
Since an inhibitory effect could be demonstrated in response to electrical stimulation of intact C. parasitica

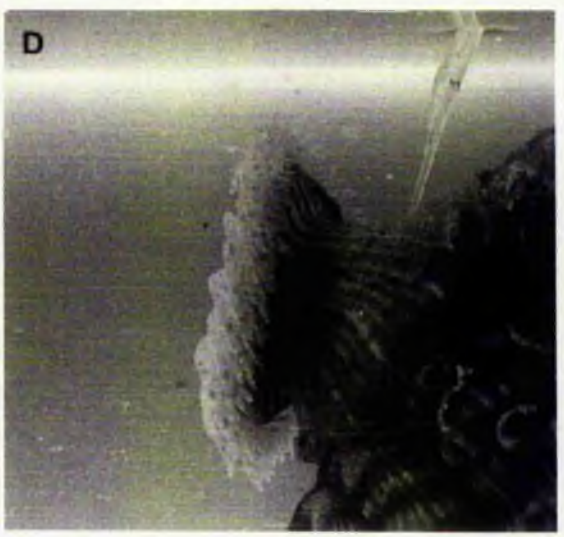
Fig. 1.4. Inhibition of slow contraction in the sphincter of intact Calliactis parasitica. The stimulating electrode, seen attached to the intact column, was used to deliver a series of electrical stimuli at a frequency of 1 shock every 5 sec. (A) Resting anemone before stimulus sequence commences. (B) Anemone contracts to the first 3-4 shocks of the series. As the sequence progresses the anemone opens (C) and the sphincter remains relaxed throughout the period of stimulation (D). Shortly after termination of the stimulus sequence the sphincter undergoes a slow contraction (E) before returning to the resting state (F). These observations indicate that the onset of slow contraction in the sphincter of the intact animal may be delayed for as long as suitable electrical stimulation is maintained.

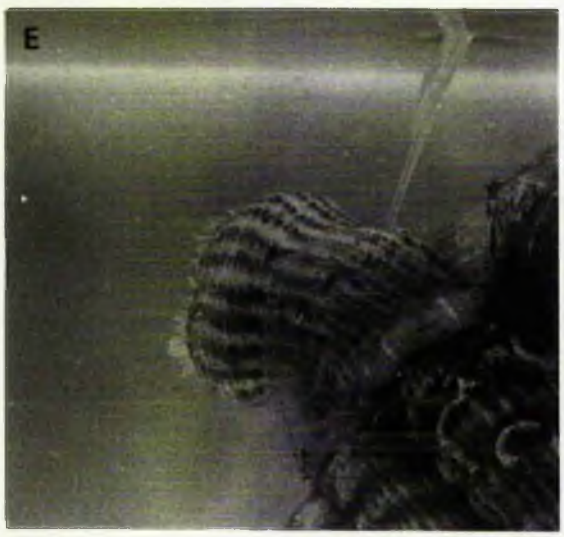
.4

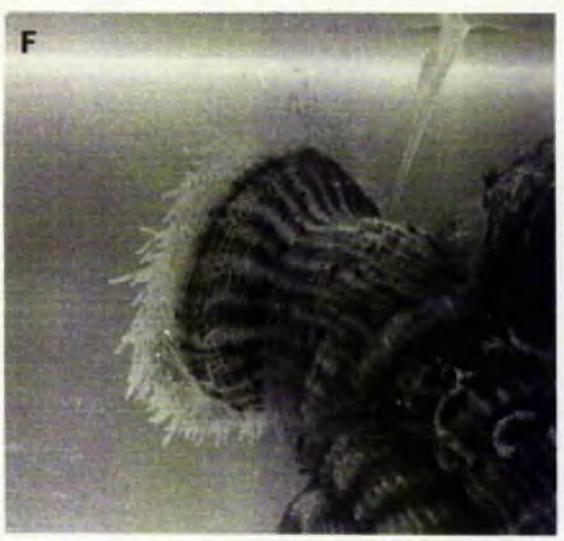












the next step was to study the effects of attaching a light isotonic lever to the sphincter of such an animal. To obtain kymograph recordings from a single muscle in an intact anemone can prove difficult, for if the separate muscle groups are not isolated from one another by operation they are bound to interfere with the desired recordings. This problem was encountered by Pantin (1935b) in his attempts to record from separate muscle groups in unoperated Calliactis. The pedal disc of the anemone was firmly attached to the substrate and the animal was hooked up in such a way that the movements of certain regions were restricted. Pantin pointed out that owing to the changes of shape that the anemone undergoes it was not possible to restrict the responses of the levers to one muscle, and from the recordings, therefore, one had to disentangle the responses of each muscle.

Observations of the intact animal during low-frequency electrical stimulation suggest that the majority of these movements result from activity of the column musculature. If the pedal disc is firmly fixed to the substrate these movements would obviously be conveyed to the unattached end of the column and hence interfere with any recordings of sphincter activity. To help overcome this problem the anemone was detached from the substrate and suspended in the sea water by a large-diameter, polythene suction electrode (internal diameter about 4 mm) attached to the mid-column region. This allows the pedal disc to move freely, and hence many of the columnar displacements are conveyed to this region rather than the partially fixed upper column region. This technique was found to greatly improve recordings of sphincter activity in the intact animal and eliminated a great deal of the columnar

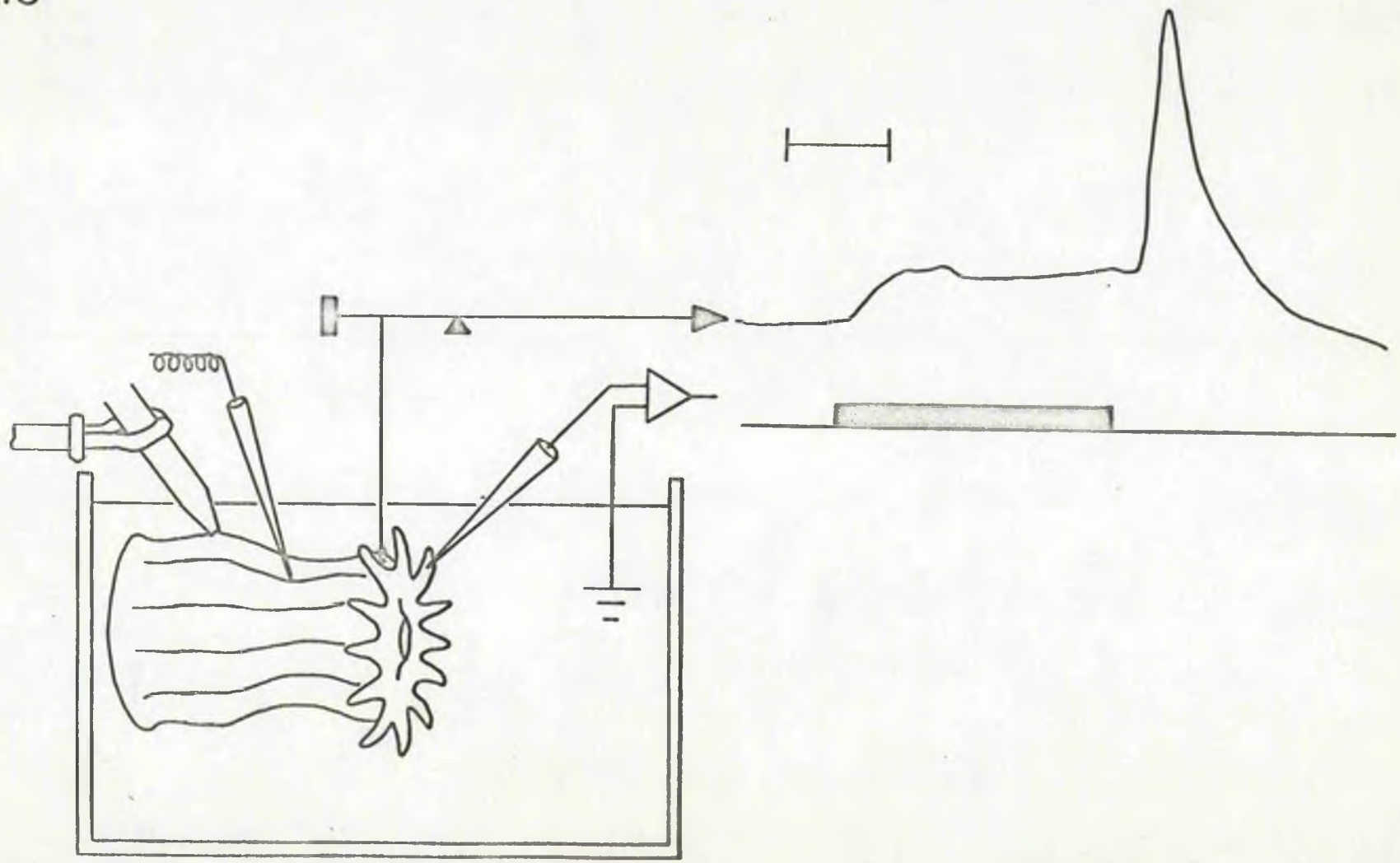
movement artifacts.

The stimulating electrode was applied to the upper column between the supporting electrode and the marginal sphincter. A thread was sewn through the thickest portion of the intact sphincter and then attached to a light isotonic lever writing on a kymograph. The animal was suspended as shown diagrammatically in Fig. 1.5. A recording electrode was attached to the mid-region of a tentacle, and 20 shocks were administered at a frequency of 1 every 8 sec. Fig. 1.5 shows that during stimulation a slight but maintained contraction appears after the first 4-5 stimuli. This could possibly be a manifestation of slow contraction occurring in muscle groups other than the sphincter as it seems to represent the slow movements noted in the preliminary experiments. At the end of stimulation, however, there is a delay of about 15 sec before a large, slow contraction of the sphincter is seen. This is not a movement due to contraction of other muscle groups as one can actually observe the sphincter undergoing this contraction, which soon dominates the recording. This experiment vividly demonstrates that during low-frequency stimulation contraction of the sphincter in the intact animal is inhibited until after the stimulation has ceased. Obviously, attaching the intact animal to an isotonic lever does not seem to affect inhibition, and therefore an attempt was made to obtain similar responses in preparations.

2) Half-Animal Preparations: The half-animal preparations require a less severe operation than that needed for isolated sphincter preparations. Ross (1957) stated that isolated sphincter rings show no spontaneous activity, whereas Ewer (1960)

Fig. 1.5. Response of sphincter in intact animal to low-frequency electrical stimulation. The anemone is suspended in the sea water by means of a wide-bore polythene suction electrode attached to the mid-column. The stimulating electrode, shown attached to the intact column, was used to deliver 20 shocks at a frequency of 1 every 8 sec. A large slow contraction of the sphincter is seen to occur only after electrical stimulation has ceased. In this and all subsequent kymograph records contraction is upwards. Time scale = 1 min.

1.5



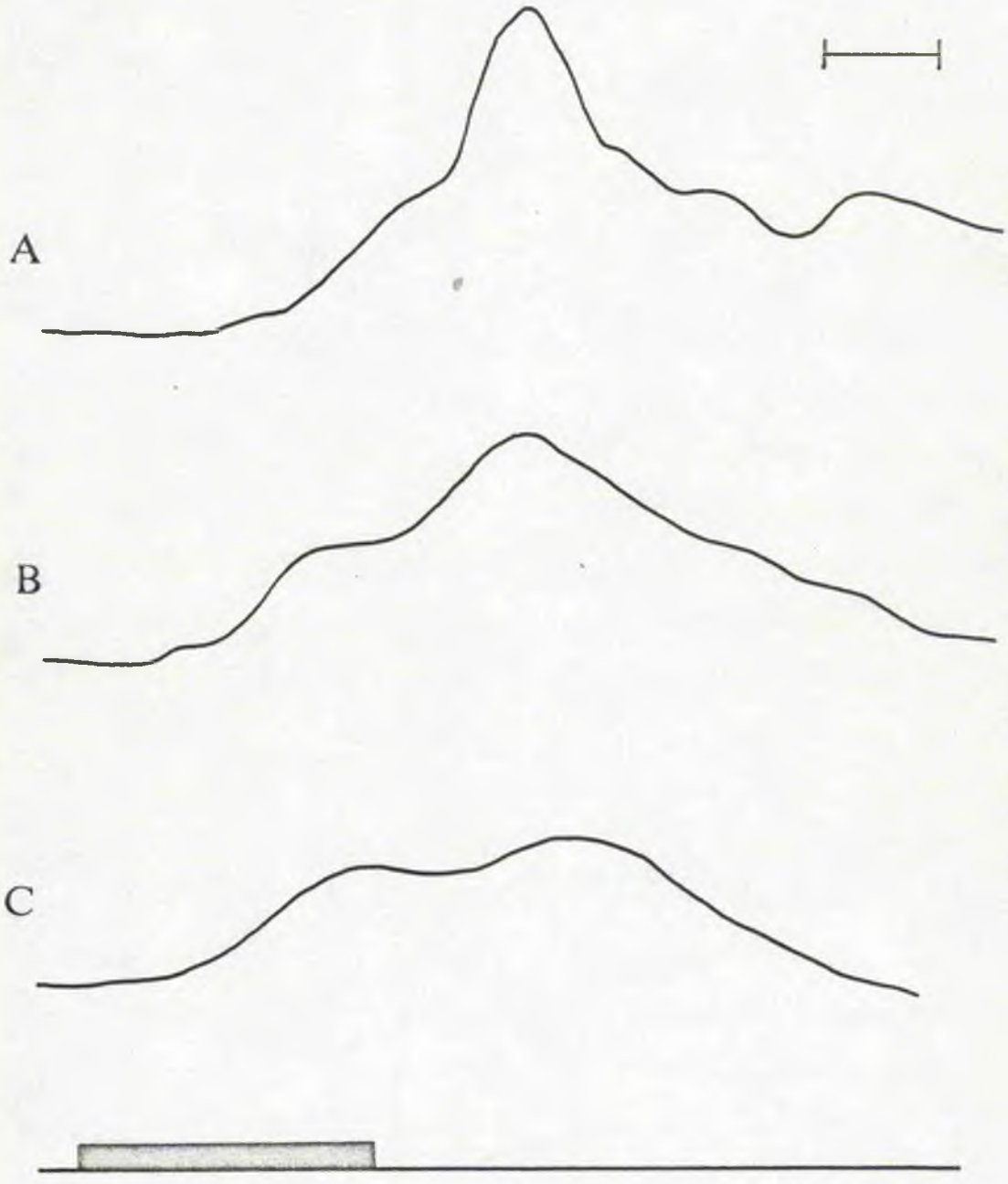
has shown that circular muscle rings from the column of Calliactis are spontaneously active. Ewer was studying inhibition of these spontaneous contractions and noted that his preparations became more excitable with age, and this often seemed to mask the inhibitory effects. If this gradual development of excitability was a reflection of the severity of the operation it was hoped that a half-animal preparation would not show these ageing effects.

Fig. 1.6 shows the effect of stimulating such a preparation at 6 hr, 24 hr and 48 hr after operation. In each case 30 shocks were applied to the intact column at a frequency of 1 every 5 sec. When the preparation is only 6 hr old (Fig. 1.6A) a similar response to that seen in the intact animal is obtained. No response seems to occur until about 15 shocks have been delivered. The trace then shows the gradual development of a slow contraction which begins to reach a plateau towards the end of the stimulus series. Shortly after cessation of stimulation a large slow contraction of the sphincter occurs and summates on the plateau formed by the previous contraction. This sphincter contraction again dominates the recording, although not as distinctly as in Fig. 1.5.

As the preparation ages it becomes increasingly difficult to separate this delayed contraction from other elements in the recording. At 24 hr (Fig. 1.6B) the initial slow contraction appears after about 10 shocks, denoting an increase in excitability of the preparation with age. The delayed component appears as a second contraction occurring after cessation of stimulation, although it tends to be less noticeable than in the 6 hr recording. After 48 hr (Fig. 1.6C)

Fig. 1.6. Increase in excitability of half-animal preparation with age. In each case 30 electrical stimuli were administered at a frequency of 1 every 5 sec. Age of preparation = (A) 6 hr old, (B) 24 hr old, (C) 48 hr old. In A the inhibited slow contraction of the sphincter is distinct from the weak preliminary contraction occurring during the period of stimulation. As the preparation ages (B) the preliminary contraction becomes more dominant until eventually (C) it almost completely masks the inhibited slow contraction. Note also that as the preparation ages the onset of the preliminary contraction occurs earlier in the stimulus sequence. Time scale = 1 min.

1.6



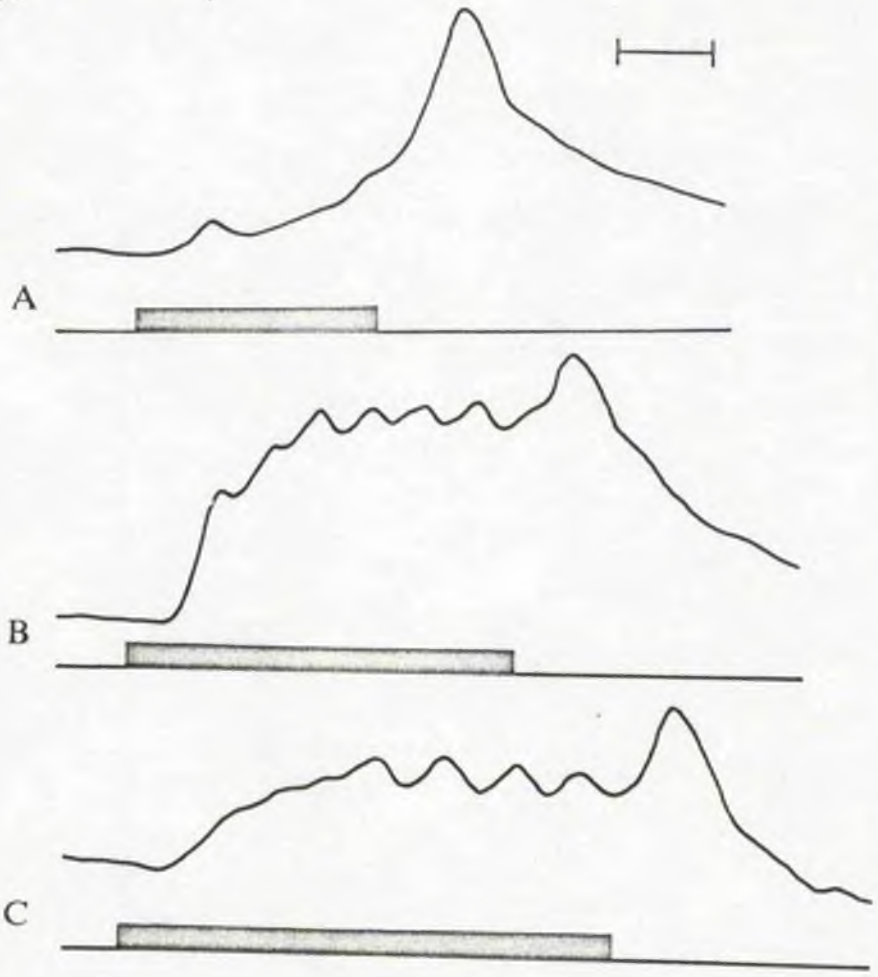
the contraction occurring during stimulation begins to dominate the recording, and the delayed component appears as a weakly developed second contraction occurring after stimulation has ceased. One cannot fail to notice the similarity between this 48 hr recording and those obtained by Ross (1957) from the isolated sphincter of Calliactis. There is a latent period of about 1 min which compares closely with that quoted by Ross for this frequency. Furthermore, he mentions that some preparations show an additional contraction (post-stimuli contraction) when the stimulus series ceases. This almost certainly corresponds to the delayed sphincter contraction noted during this study, although as Ross points out such a contraction could be due to the existence of other muscle groups in the submarginal region. The isolated sphincter preparations employed by Ross were allowed to recover for 2-3 days and they give results bearing a striking resemblance to the recordings obtained in this study from preparations of the same age.

The effects of different frequencies of stimulation on a preparation 24 hr old are shown in Fig. 1.7. A consistent feature of the recordings is the occurrence of the delayed sphincter contraction shortly after cessation of stimulation. The nature of the contractions occurring during stimulation, however, seems to change with frequency. At a frequency of 1 shock every 5 sec (Fig. 1.7A) these contractions are much reduced and gradually increase during stimulation. A vastly different record is obtained at a frequency of 1 shock every 8 sec (Fig. 1.7B). The initial contraction commences after 3-4 stimuli and develops rapidly until a plateau is reached. A series of small contractions seems to be superimposed on

Fig. 1.7. Effect of stimulation at different frequencies in a preparation 24 hr old. In each case 30 electrical stimuli were administered at frequencies of : (A) 1 every 5 sec, (B) 1 every 8 sec, (C) 1 every 10 sec. The inhibited slow contraction of the sphincter is conspicuous in each recording. The nature of the preliminary contraction occurring during stimulation can be seen to vary with frequency. In A this contraction is weakly developed and gradually builds up during the stimulus sequence. In B and C the contraction commences earlier than in A and develops rapidly until a plateau is reached. The different nature of the preliminary contractions at varying frequencies would suggest that they are produced by muscle groups other than the sphincter. Time scale = 1 min.

Fig. 1.8. Showing duration of inhibitory effect. A half-animal preparation was electrically stimulated at a frequency of 1 shock every 10 sec for 35 min. Note that even after such a long period of stimulation the inhibited slow contraction of the sphincter (denoted by the dot) still occurs after the stimulus sequence has ceased. Time scale = 5 min.

1.7



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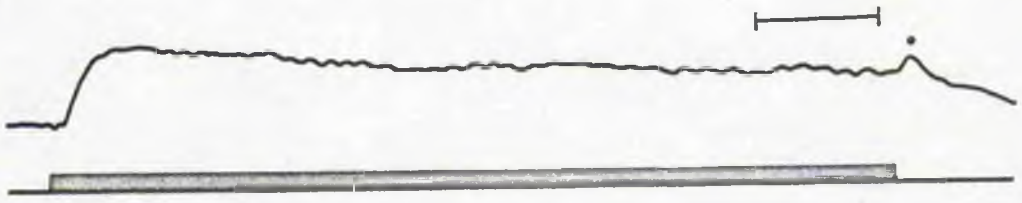


this plateau and these continue throughout the period of stimulation. At a stimulus frequency of 1 shock every 10 sec (Fig. 1.7C) a similar picture emerges. The plateau of the initial contraction is reached over a longer time period, although once again the contraction commences after 3-4 stimuli. The height of the plateau relative to the resting length appears to be reduced and the frequency of the series of superimposed contractions is lower.

These results indicate that the initial components may be due to the activation of muscle groups other than the sphincter. Pantin (1935b) noted that stimuli given at a frequency between 1 every 10 and 1 every 6 sec called forth slow contractions of the circular muscles, and at a frequency of 1 every 3 sec a slow contraction of the parietal muscles ensued. It is, therefore, possible that the components seen during stimulation at frequencies of 1 every 8 and 1 every 10 sec represent contractions of the circulars. The different type of contraction seen at a stimulus frequency of 1 every 5 sec could represent activity in the parietals. The fact that in this case the contraction appears weak could be explained on the basis that these muscles act at 90^{o} to the sphincter. In addition, stimulation at 1 every 5 sec may not be the most effective frequency for the parietals for they contract with full strength between stimulus frequencies of 1 every 2 and 1 every 3 sec (Pantin, 1935b).

The delayed contraction of the sphincter is quite obvious at these different frequencies and is never seen to occur during a stimulus series. This suggests that the delay is not due to latency of response for if such were the case the contraction would always commence after a fixed number of

stimuli. This does not occur, for the contraction may be delayed merely by continuing the stimulation sequence, thereby implying that the stimuli are capable of inhibiting the slow contraction of the sphincter muscle.

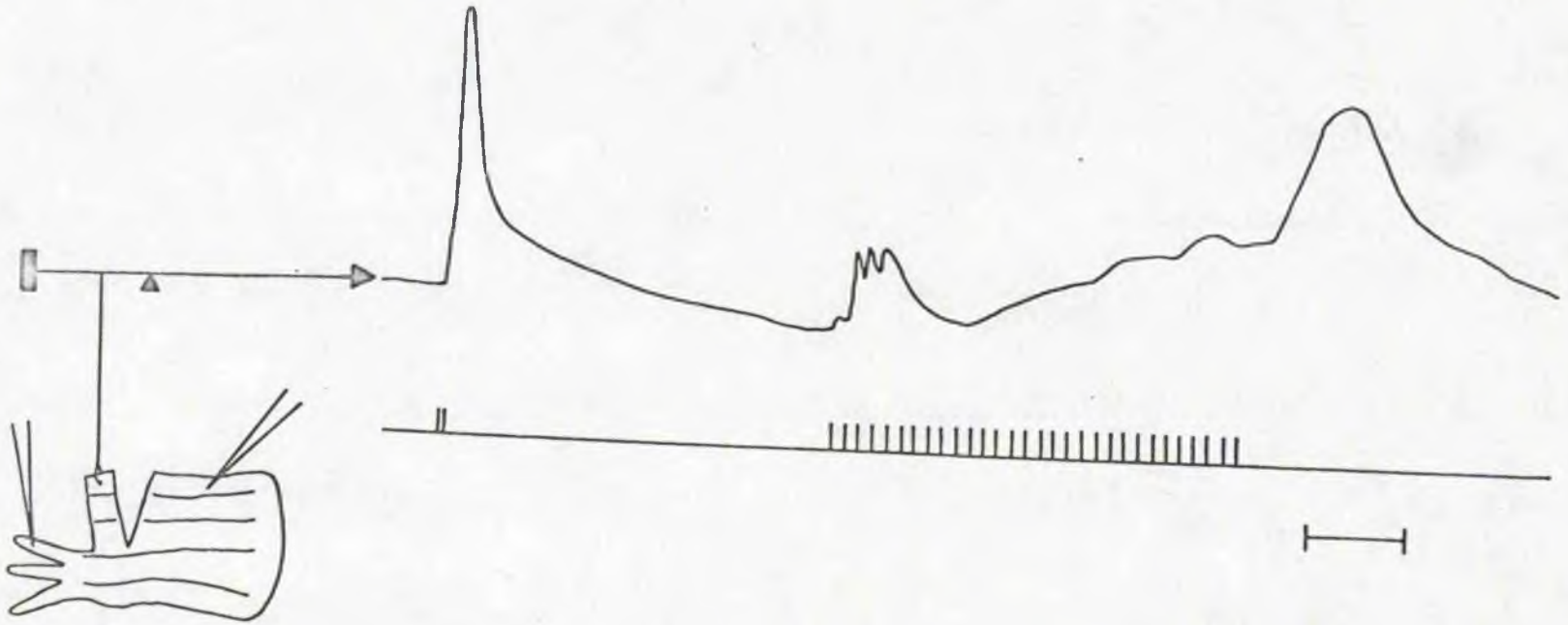
The inhibitory effect can be maintained over remarkably long time periods. Fig. 1.8 shows the effect of stimulating a preparation (30 hr old) at a frequency of 1 every 10 sec for 35 min (the experiment was terminated owing to lack of space on the kymograph). The slow sphincter contraction still occurs after cessation of stimulation. This demonstrates that as long as stimulation is maintained the contraction may be inhibited almost indefinitely.

3) Sphincter Preparations: To further confirm that inhibition occurs in the sphincter itself a series of experiments was carried out on partially isolated sphincter preparations. These were obtained by further dissection of half-animal preparations (see Materials and Methods). Both loops and strips of sphincter were examined, and they seemed to show no difference in activity. Because of their geometry the strips produced larger contractions on the kymograph recordings, so these were used in the majority of the following experiments.

Fig. 1.9 gives a diagrammatic representation of the sphincter strip experiments. A recording electrode is shown attached to a tentacle to monitor the electrical activity induced by the stimulating electrode (attached to the intact column). The rapid twitch-like contraction at the beginning of the trace shows the response of the sphincter to 2 shocks only 500 msec apart. The typical quick response

Fig. 1.9. Responses of the sphincter strip preparation to electrical stimulation of the intact column. Firstly 2 shocks at an interval of 500 msec were administered to evoke a typical facilitated fast contraction in the sphincter. The sphincter strip was then allowed to return to its resting length and a series of 30 shocks at 1 every 8 sec was applied. Note the small facilitated contractions occurring early in the stimulus sequence, an effect often observed in rested preparations. Later in the sequence a weak preliminary contraction develops, and after stimulation has ceased the inhibited slow contraction of the sphincter dominates the recording. Time scale: = 1 min.

1.9



(Pantin, 1935a; Ross, 1957) is seen to occur on the second stimulus. This indicates that the machinery responsible for fast contractions of the sphincter is present in this type of preparation. Once the sphincter had returned to its original length a series of 30 shocks at a frequency of 1 every 8 sec was applied. Note that a small twitch occurs at the second stimulus followed by a facilitated contraction at the third. This is succeeded by smaller, slightly summated contractions at the fourth and fifth stimuli before the sphincter returns to its original length. As stimulation is maintained, however, a gradual slow contraction begins to commence almost immediately, and this continues until the end of stimulation. Shortly after cessation of the stimulus series the sphincter strip gives a large slow contraction which appears identical to the delayed contractions seen in the previous experiments. This demonstrates that the inhibitory effect still exists in strip preparations and strongly supports the conjecture that inhibition acts on the sphincter muscle itself.

The series of twitches seen at the start of the stimulus sequence are often noticed when a preparation that has been resting for several hours is given its first series of low-frequency shocks. These twitches are then no longer encountered during subsequent experiments, unless the animal is allowed to rest for several hours once again. It would seem, therefore, that the resting preparation gradually develops excitability to such an extent that fast, facilitated twitches can occur to the first few shocks in a low-frequency series. A process of adaptation then seems to occur, resulting in a loss of this hypersensitivity. The mechanisms involved in

such a response are not known, but these twitches probably correspond to the retractions occurring to the first few stimuli in intact animals (see Fig. 1.4B). This would suggest that the preparations are not giving artificial responses due to some side-effect of the operation.

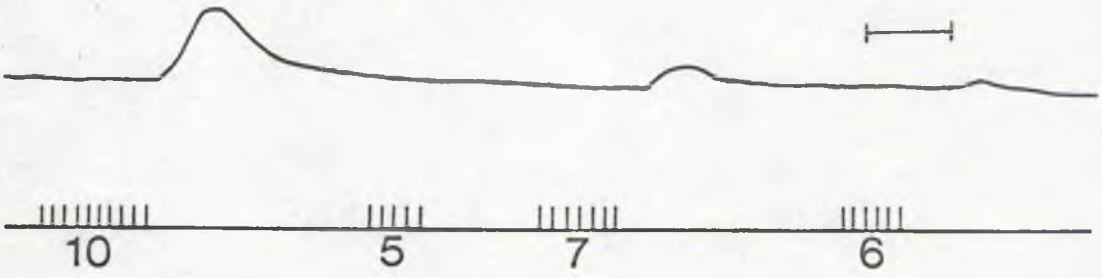
The minimum number of shocks required to produce a slow contraction of the sphincter was determined for a stimulus frequency of 1 every 8 sec. This is shown in Fig.1.10 where successive sequences of stimulation, each with a different number of shocks, were applied to the preparation. A contraction is seen after 10 shocks but none after 5. A small contraction occurs to 7 shocks and this is only just discernible after 6, which may be regarded as the minimum number of shocks required to evoke a visible slow contraction. Ross (1957) obtained similar results in isolated sphincter loop preparations, and also noted that stimuli in excess of this number add to the size of contraction. This is confirmed in Fig. 1.11 where the delayed contraction of the sphincter increases in size with increase in number of stimuli at a constant frequency. The remarkable consistency with which this response may be called forth in different preparations leads one to accord with Ross' statement that "one of the most impressive features of the slow contraction ..... is the regularity with which the response appears after a certain number of stimuli are given, almost as if a counter of some kind were involved".

Although this type of preparation eliminates a great deal of the extraneous movements, the presence of additional muscle elements in the sphincter strip cannot be avoided. Robson (1965) has shown that endodermal circular

Fig. 1.10. Minimum number of shocks required to evoke a slow contraction of the sphincter. Electrical stimulation at a frequency of 1 shock every 8 sec. The record indicates that a minimum of 6 stimuli at this frequency are required to produce a visible contraction. Time scale = 1min.

Fig. 1.11. To show the relationship between magnitude of slow sphincter contraction and number of applied stimuli. Frequency of stimulation in each case = 1 shock every 8 sec. Number of applied stimuli: (A) 30, (B) 10, (C) 7. The size of the inhibited slow contraction of the sphincter increases with the number of applied stimuli. Note that a preliminary contraction begins to develop in A during the stimulus sequence. Time scale = 1 min.

1.10



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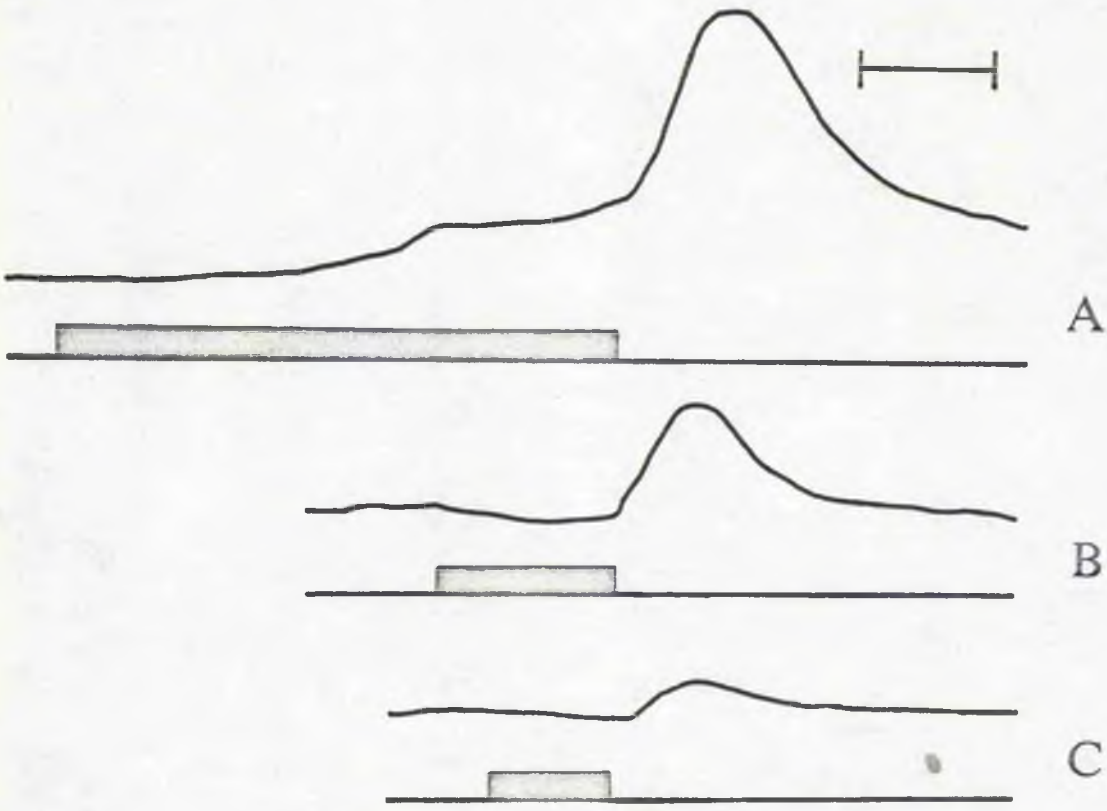
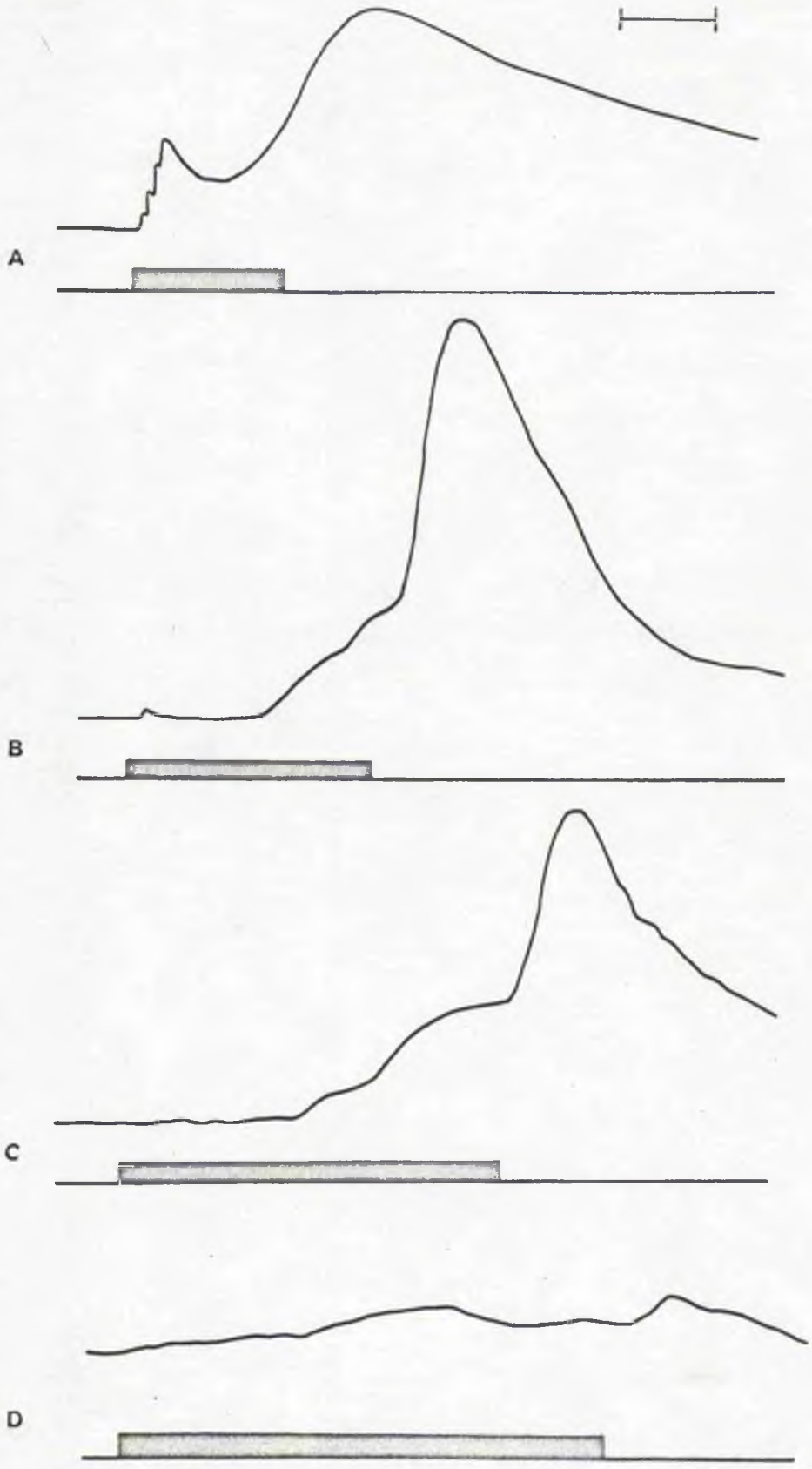


muscles line the entire column and are separated from the sphincter by mesogloea at least 100μm thick. Such elements would obviously be included in the sphincter strip, and one would expect that their activity could be demonstrated in an ageing preparation by stimulation at different frequencies, in a similar manner to that described in thehalf-animal experiments (Fig. 1.7). This is shown in Fig. 1.12 where 30 stimuli over a wide range of frequencies are applied to a preparation 3 days old. At a frequency of 1 every 3 sec (Fig. 1.12A) fast facilitated contractions occur on stimuli 2 to 5. The muscle then relaxes slightly until a large slow contraction commences during the period of stimulation. Allowing for the fact that facilitation decays less rapidly at the relatively low temperatures encountered in these experiments one can interpret the fast contractions as an activation of the quick response at this frequency. Ross (1957) was able to obtain this type of response at a frequency of 1 every 3.6 sec at temperatures similar to these. The quick response seems to fail after the fifth stimulus and a large slow contraction takes over, appearing to mask all signs of the delayed contraction. One explanation for this may be that the frequency is too high for the inhibitory effect to work, and the excitatory response consequently dominates the recording.

At a frequency of 1 every 5 sec (Fig. 1.12B) the inhibitory effect seems to be evident, as there is a large slow contraction occurring after cessation of stimulation. A similar response occurs at 1 every 8 sec (Fig. 1.12C), and in both recordings a gradual slow contraction appears during stimulation after about 15 stimuli. As in the half-animal preparations it is possible that this may be due to activation

Fig. 1.12. Effect of different frequencies of stimulation in an aging sphincter strip preparation. Total of 30 stimuli administered in each case. Frequency of stimulation: (A) 1 shock every 3 sec, (B) 1 every 5 sec, (C) 1 every 8 sec, (D) 1 every 10 sec. Note the occurrence of fast facilitated contractions in A, followed by a large slow contraction which soon dominates the recording. There seems to be no sign of an inhibitory response at this frequency. In all the other recordings the inhibited slow contraction is evident. As in the half-animal preparations, the nature of the preliminary contraction occurring during stimulation seems to vary with the frequency of the applied stimulus. This indicates that muscles other than the sphincter are present in the sphincter strip. Time scale = 1 min.

1.12



of the endodermal circulars lining the sphincter strip. At a frequency of 1 every 10 sec (Fig. 1.12D) both the initial and delayed components are present, but reduced. In this particular case it is possible that the frequency is too low to call forth strong contractions of either muscle group, although the presence of a small delayed contraction indicates that the inhibitory effect is still active at low frequencies.

A close examination of these records reveal that the interval between the final stimulus of the series and the onset of delayed contraction varies with frequency. This interval presumably represents the time taken for the inhibitory effect of the preceding pulse to decay. A series of experiments were devised to find the optimal interval between successive stimuli required to give a maximal duration of inhibition. In all the experiments a preliminary 20 shocks at a frequency of 1 every 8 sec were administered. The interval between the 20th stimulus and the onset of delayed contraction was noted for each preparation. This represents the decay time of the inhibitory effect produced by the 20th stimulus. Once this had been established, an additional shock was applied at varying intervals after the end of the series in order to increase the time period between the 20th stimulus and the onset of contraction. This would occur because the 21st pulse would produce an additional inhibitory effect.

Table 1.1 gives typical values for such a series of experiments carried out on a single preparation. The accompanying graph (Fig. 1.13) is constructed from the data quoted in this table and plots the increase in contraction delay against the interval of the 21st pulse. It shows quite

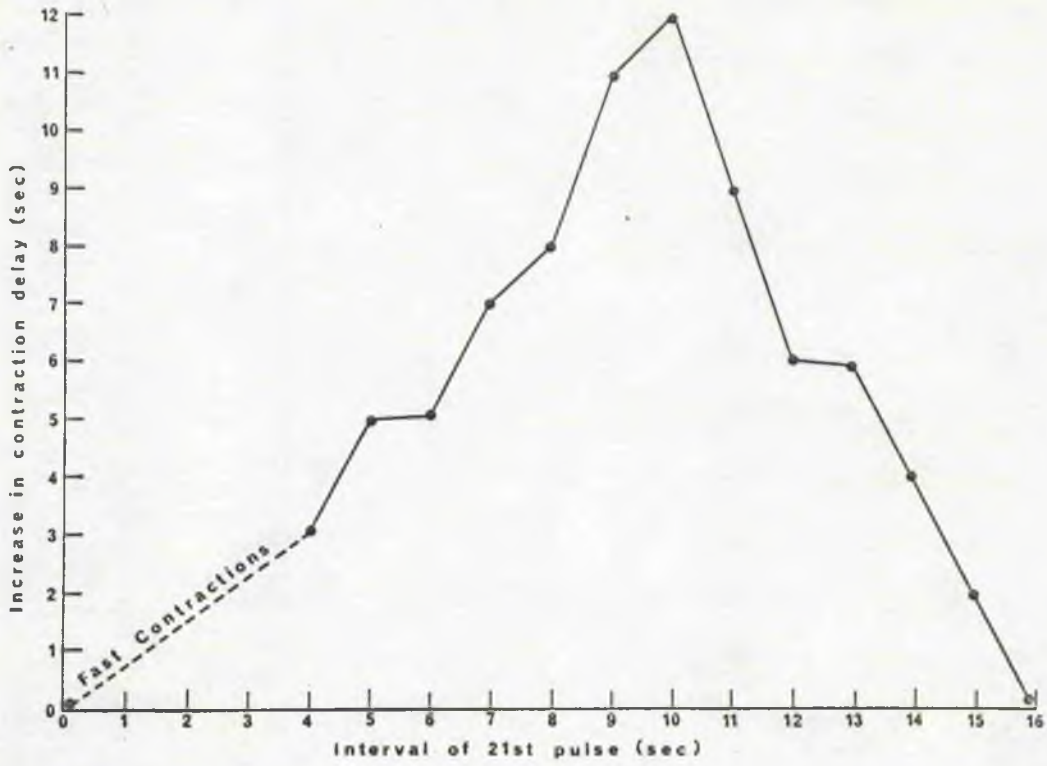
Table 1.1. Typical data from a series of experiments to determine the relationship between pulse interval and duration of inhibition. In each trial a series of 20 stimuli at a frequency of 1 shock every 8 sec was initially applied to the preparation. A 21st shock was then administered at varying intervals from the time of the 20th shock. The subsequent variation in the delay of the inhibited slow contraction was then plotted against the time interval of the 21st shock to determine the optimum pulse interval required to elicit a maximal duration of the inhibitory effect (see Fig. 1.13).

Interval of 21st pulse (sec)	Time from 20th pulse to onset of contraction (sec)	Increase in contraction delay (sec)
0	16	0
4	19	3
5	21	5
6	21	5
7	23	7
8	24	8
9	27	11
10	28	12
11	25	9
12	22	6
13	22	6
14	20	4
15	18	2
16	16	0

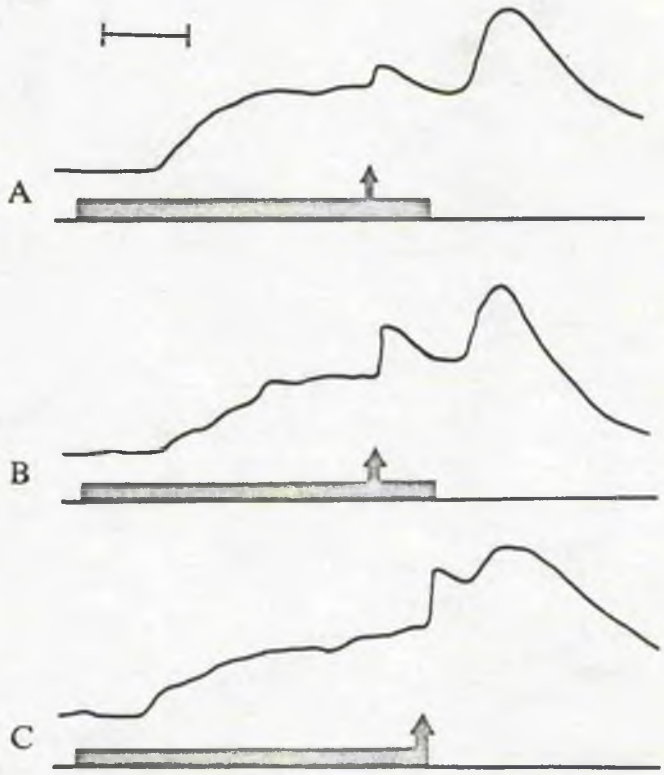
Fig. 1.13. Graph showing the relationship between pulse interval and contraction delay. Values taken from Table 1.1. At a pulse interval of 9-10 sec a maximum increase in contraction delay is obtained. This indicates that a maximal duration of the inhibitory effect is obtained at stimulus frequencies of 1 pulse every 9-10 sec.

Fig. 1.14. Fast contractions and the inhibitory response. A low-frequency series of stimuli was applied to the preparation in order to induce the inhibitory response in the sphincter. High-frequency stimuli were intercalated during this series (at the points marked by the arrows) in order that the effects of the inhibitory response on fast contractions might be observed. In all cases the inhibitory stimuli were applied at a frequency of 1 shock every 8 sec. (A) A single shock was intercalated 500msec after the 25th shock, and the low-frequency series was then continued for a further five stimuli. Note the fast contraction of the sphincter occurring on the intercalated shock. (B) Stimulus sequence as in A, but two high-frequency shocks intercalated after the 25th shock. Note the typical summated fast contractions. (C) Effect of administering two high-frequency shocks at end of low-frequency series. Once again, summated fast contractions occur on these stimuli and tend to mask the onset of the inhibited slow contraction. These experiments indicate that the inhibitory response acts only on slow contractions of the sphincter and appears to have no effect whatsoever on fast contractions. Time scale = 1 min.

1.13


Increase in contraction delay (sec)
Fast Contractions
Interval of 21st pulse (sec)

1.14


A
B
C

clearly that the maximum contraction delay occurs at a pulse interval of 9-10 sec, and on either side of this value there is a decrease in delay. Responses at pulse intervals below 4 sec were not recorded since these elicit quick facilitated contractions which make it impossible to determine the onset of the slow contraction. The significance of these results will be discussed later.

One question that arises from these observations is this: does the inhibitory effect act on slow sphincter contractions alone, or does it also influence the fast contractions? This problem is easily resolved by intercalating 2 or 3 high-frequency stimuli during the low-frequency series. In Fig. 1.14A a series of 25 shocks at a frequency of 1 every 8 sec were administered, the 26th shock was intercalated 500 msec later, and the low-frequency series then continued for five more shocks. A fast contraction is seen to occur at the 26th shock and this summates onto the initial slow contraction. This indicates that the fast machinery of the sphincter is not inhibited by the low-frequency stimulation. The fast response does not seem to release the slow machinery from inhibition, however, for as long as inhibitory stimulation is maintained the delayed slow contraction will not occur.

Fig. 1.14B shows a similar experiment in which both the 26th and 27th shocks were intercalated, each at an interval of 500 msec. Typical facilitated fast contractions occur to both stimuli, and these summate on one another as shown. This demonstrates that the quick response still shows facilitation and summation, and in this respect appears to be completely unaffected by the low-frequency series. Once more, a delayed slow contraction occurs on cessation of the stimulus

sequence. Fig. 1.14C shows the effect of applying 2 shocks at high frequency (500 msec intervals) at the end of a low-frequency series. The fast facilitated contractions occur immediately on these stimuli and tend to mask the onset of the delayed slow contraction. It is for this reason that Table 1.1 does not record the increase in slow contraction delay for frequencies higher than 1 every 4 sec.

These experiments provide convincing evidence that the inhibitory effect does not seem to act on fast contractions of the sphincter. This implies that the fast and slow machinery in the sphincter are functionally separate in this respect. The observation that in the intact animal mechanically induced inhibition seems to prevent fast retractions could be explained in terms of a rise in sensory threshold, rather than a direct inhibition acting on the fast component itself. Such a mechanism has been proposed to explain the inhibitory effects observed in Stomphia coccinea during swimming, or when food juice contacts the tentacular crown (Ross & Sutton, 1964b).

The very distinctive type of contraction associated with the quick response (see Fig. 1.14) suggests that the slow contraction occurring during stimulation is not due to a gradual activation of fast components in the sphincter muscle. This would imply that these initial contractions are due to some other muscle group in the sphincter strip and evidence has been given to suggest that the endodermal circulars might be responsible. To investigate this more thoroughly several experiments on double preparations were carried out.

4) Double-Preparations: A strip of circular muscle was

partially isolated from the mid-column region of a sphincter preparation. The base of the strip was left attached to the column and its free edge was attached by thread to a light isotonic lever. The section of column between the sphincter and mid-column strips was trimmed away to produce the type of preparation shown diagrammatically in Fig. 1.15. A recording electrode is shown attached to a tentacle and the stimulating electrode is on the intact lower column. A series of 30 shocks at a frequency of 1 every 8 sec was administered to the preparation and the activity in the two muscle strips recorded simultaneously. Both records clearly show an almost identical slow contraction which begins after about 7 shocks and continues throughout the stimulus sequence. This strongly suggests that low-frequency stimulation calls forth a slow contraction of the circulars which is maintained as long as stimulation continues. This confirms Pantin's (1935b) observations that slow contractions of the column circulars are evoked by stimuli at frequencies between 1 shock every 10 sec and 1 every 6 sec.

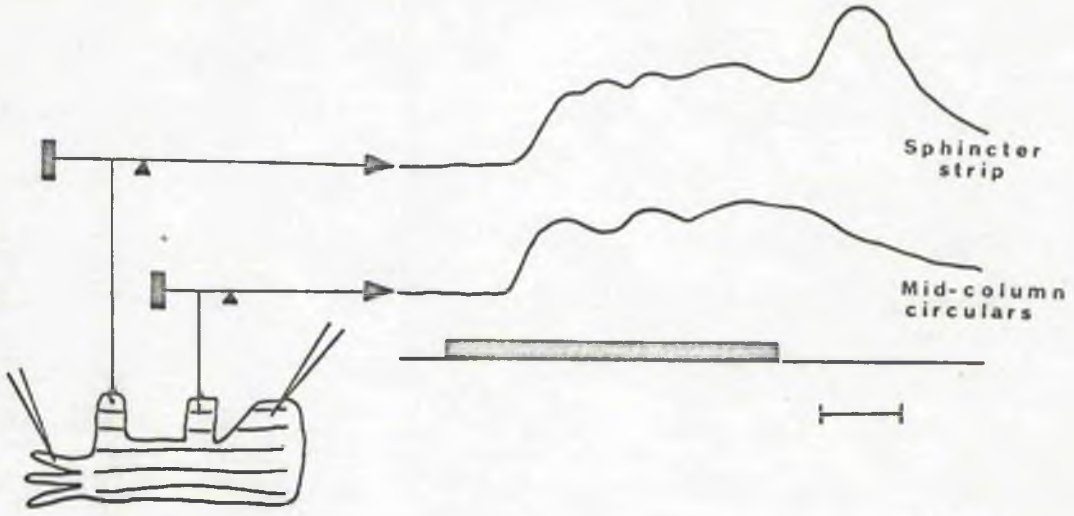
At the end of stimulation the sphincter strip produces a delayed slow contraction not seen in the mid-column strip. These results seem to indicate that the initial contractions seen in all the previous recordings are due to the endodermal circulars which line the sphincter strip. The inhibited slow contraction occurring after cessation of stimulation, however, seems to be a response of the sphincter muscle itself.

In order to verify these observations an additional experiment was carried out on a similar preparation. Fig. 1.16A shows the identical experiment to that seen in Fig. 1.15. Note

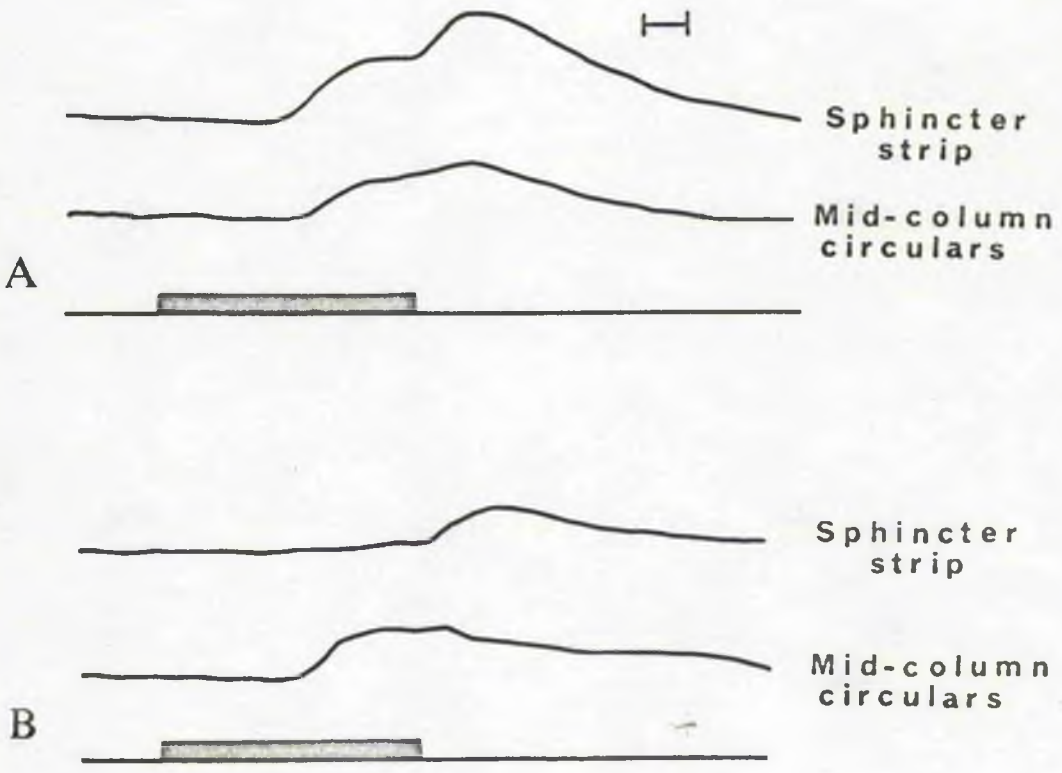
Fig. 1.15. Response of double preparation to electrical stimulation. Recording electrode shown attached to a tentacle, stimulating electrode on the intact lower column. 30 shocks at 1 every 8 sec were applied to the preparation. The sphincter strip and the mid-column circulars both respond with almost identical slow contractions which commence after about 7 shocks and continue throughout the stimulus sequence. Only the sphincter strip, however, undergoes a delayed slow contraction after stimulation has ceased. This indicates that the inhibitory response acts on the sphincter muscle alone, and the slow contraction occurring during stimulation is due to the circular muscles which line the sphincter strip. Time scale = 1 min.

Fig. 1.16. Response of double preparation to electrical stimulation: (A) Sphincter strip unscraped. (B) Sphincter strip scraped to remove endodermal circulars. Stimulation parameters as in Fig. 1.15. Note that in A the sphincter strip (upper trace) undergoes a slow contraction during stimulation followed by the inhibited slow contraction. In B the same strip shows no sign of the preliminary slow contraction but still undergoes the inhibited slow contraction. This provides convincing evidence that the endodermal circulars in the spincter strip are responsible for the preliminary slow contraction. Time scale = 1 min.

1.15


Sphincter strip
Mid-column circulars

1.16


Sphincter strip
Mid-column circulars
A
Sphincter strip
Mid-column circulars
B

once again the contraction of the column circulars during stimulation, as seen in both recordings, and the inhibited slow contraction seen only in the recording from the sphincter region. The endodermal surface of the sphincter strip was then scraped very carefully, in an attempt to remove the attached layer of column circular muscle, and the preparation was then left to recover for 18 hr. The same stimulus sequence was then administered to the modified preparation and the response seen in Fig. 1.16B was obtained. Although the severity of the operation tends to decrease the responsiveness of the sphincter (possibly due to removal of part of its musculature) the recording from this region shows no obvious contraction during stimulation, unlike the mid-column recording. The inhibited slow contraction of the sphincter is still present, however, indicating that this effect does not disappear with removal of the endodermal circulars. Histological examination of the sphincter strip used in this experiment confirmed the presence of both sphincter and endodermal circular muscles in the unscraped strip. In the scraped strip, however, it was found that the operation had removed the circulars entirely, leaving the sphincter as the only remaining muscle element.

These observations strongly endorse the conjecture that slow contraction of the sphincter can be actively inhibited by low-frequency electrical stimulation.

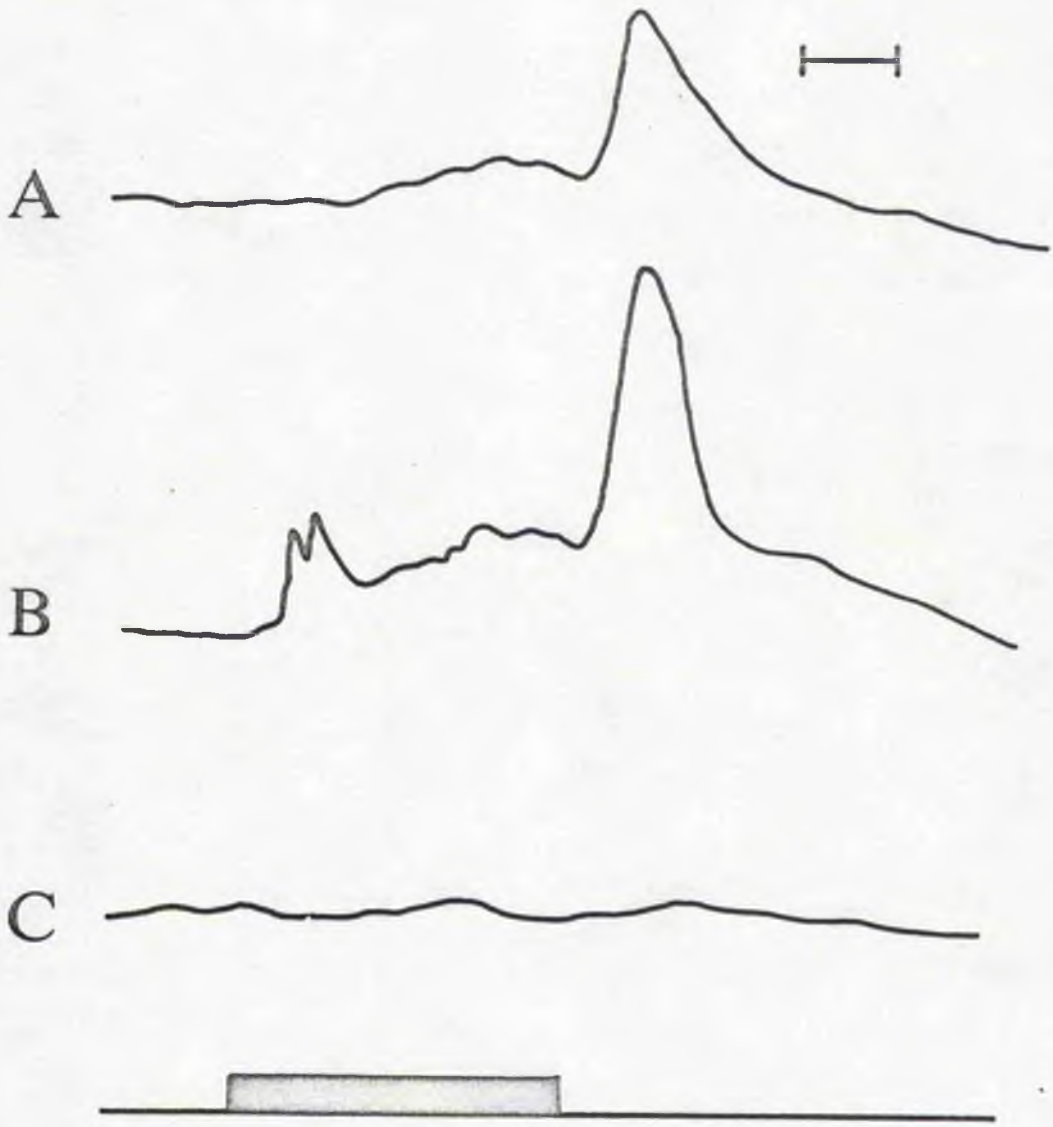
5) Conduction Systems Involved: In all the previous experiments preparations were stimulated at an intensity above SS2 threshold as this ensures that all three conduction systems are being stimulated. This, of course, raises the question of which conduction system mediates the inhibitory response.

Using the flap technique described earlier it is possible to stimulate the nerve-net and SS1 separately. As yet there is no known method of stimulating the SS2 alone (McFarlane, 1973a) and thus one has to determine its actions indirectly. Fig. 1.17A shows the effect of stimulating all three conduction systems for 25 shocks at a frequency of 1 every 8 sec. A typical response is obtained and the delayed slow contraction is quite obvious. In Fig. 1.17B the same sequence of stimulation is applied to the nerve-net alone. During stimulation the sphincter seems relatively excitable and a few fast contractions are observed. At the end of stimulation, however, the inhibited slow contraction dominates the recording and this would indicate that the inhibitory response is mediated (either directly or indirectly) by the through-conducting nerve-net. McFarlane (1973a) has shown that a burst of spontaneous nerve-net pulses often occurs during quiet periods of SS2 activity. The increased excitability of the preparation may, therefore, be due to the fact that the SS2 is not being excited during stimulation. In the following section evidence is presented to offer a possible explanation for the fast contractions. The response shown in Fig. 1.17C shows that the SS1 has no apparent effect on sphincter activity. This would be expected with a system which is believed to be located in the ectodermal epithelium (McFarlane, 1969b), for the sphincter appears to have no links with the ectoderm (Robson, 1965).

The fact that the inhibitory response can be evoked by stimulation of any region of the preparation suggests that it is not mediated by some local system, but resides in the through-conduction system. If the stimulating electrode is

Fig.117. Response of sphincter preparation to stimulation of different conduction systems. In all cases 25 shocks at a frequency of 1 every 8 sec were administered (using the flap technique described earlier) in order to stimulate: (A) the nerve-net, SS1 and SS2, by attaching the stimulating electrode to the intact column, (B) the nerve-net alone, by low-intensity electrical stimulation of the mesogloea beneath the flap cut in the column, (C) the SS1 alone, by electrical stimulation of the flap at voltages above SS1 threshold. Note that the inhibited slow contraction is present in A and B but absent in C. This indicates that the inhibitory response is mediated by the through-conducting nerve-net. The contractions observed during nerve-net stimulation in B may be due to the fact that the SS2 is not being stimulated in this case. Time scale = 1 min.

1.17



attached to the sphincter strip itself it is not possible to obtain an excitatory response in the absence of inhibition. This would suggest that the inhibitory machinery is not located at some remote junction in the through-conducting nerve-net. If an inhibitory synapse were present, then it is likely to be located at the neuromuscular junction itself.

In relation to this it is interesting to note that reciprocal inhibition between diametrically opposed parieto-basilars in Stomphia coccinea (Hoyle, 1960) does not seem to occur at the neuromuscular junction, for the inhibited muscle can be excited by locally applied electrodes. Until direct evidence from intracellular recordings can be obtained from the elements concerned it is difficult to see how the problem of locating the seat of inhibition in Calliactis may be resolved.

Spontaneous Activity

Spontaneous slow contractions of the marginal sphincter cause unstimulated, intact Calliactis to close up from time to time (Needler & Ross, 1958). In the majority of animals this was found to occur about once an hour. The situation in the isolated sphincter ring seems to be different, however, for it is characterised by the absence of rhythmical activity (Ross, 1957). This would suggest that isolation of the sphincter destroys or inhibits spontaneous nerve-net activity, perhaps as a result of separation from possible pacemaker sites (McFarlane, 1973a). The spontaneous activity of the preparations employed during this study was investigated in the hope of throwing some light onto the mechanisms which

control and coordinate muscular activity. The partially isolated sphincter preparations seem to retain the spontaneous activity seen in intact animals, and these were employed in conjunction with studies on half-animal preparations.

It is difficult to monitor spontaneous electrical activity in the intact animal because the electrodes are invariably thrown off during the rapid movements undertaken by the anemone. In addition, the use of two recording electrodes increases the difficulty in maintaining contact with the tentacles for the long periods required in this type of work (4-5 hr). The preparations, however, produced virtually no strong displacements (possibly owing to a combination of judicial pinning and the fact that the sphincter is unable to fold inwards) and these proved the most useful for long-term monitoring.

A Servomex low-frequency waveform generator was employed to produce a series of successive sweeps, one beneath the other, on the screen of the storage oscilloscope. This was achieved by feeding a single, long-duration (about 160 sec) sawtooth waveform into a d.c. - coupled channel of the differential amplifier attached to the oscilloscope. The beam was thus gradually displaced from the top to the bottom of the screen and a sequence of stored sweeps, over a time period of upto 2 min 40 sec, could be photographed in a single frame. Recording at slow-sweep speeds is only possible with a high signal to noise ratio, otherwise the pulses cannot be readily identified.

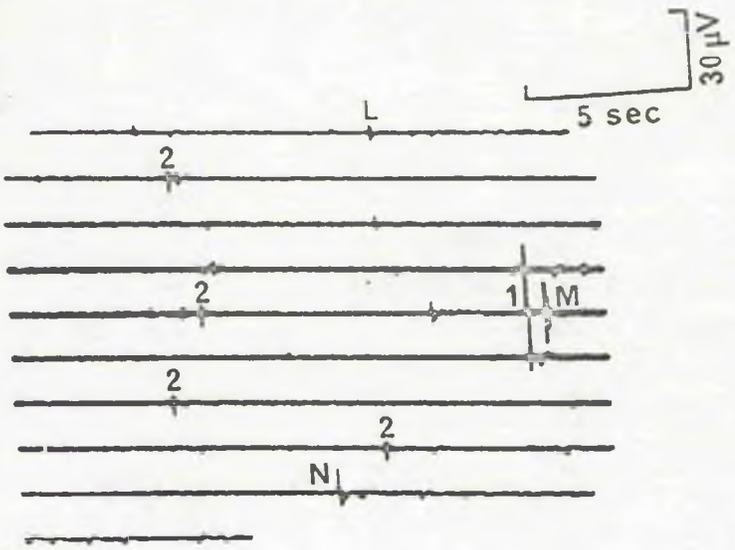
A monitored sequence is seen in Fig. 1.18, which shows a wide range of pulse types commonly observed in recordings of spontaneous electrical activity. One can identify

Fig. 1.18. Monitored sequence of spontaneous electrical activity in C. parasitica. A number of commonly occurring pulse types may be seen in this particular sequence. A local movement artifact (L) is seen at the beginning of the sequence. A number of SP2s are present (2). These display a characteristic downward deflection in this particular recording. A single large SP1 is also recorded (1) and this is succeeded by a pulse representing a possible local muscle action potential (M). Near the end of the sequence a single pulse in the nerve-net may be seen (N). All these pulse types have characteristic shapes that enable the experienced observer to distinguish one from the other.

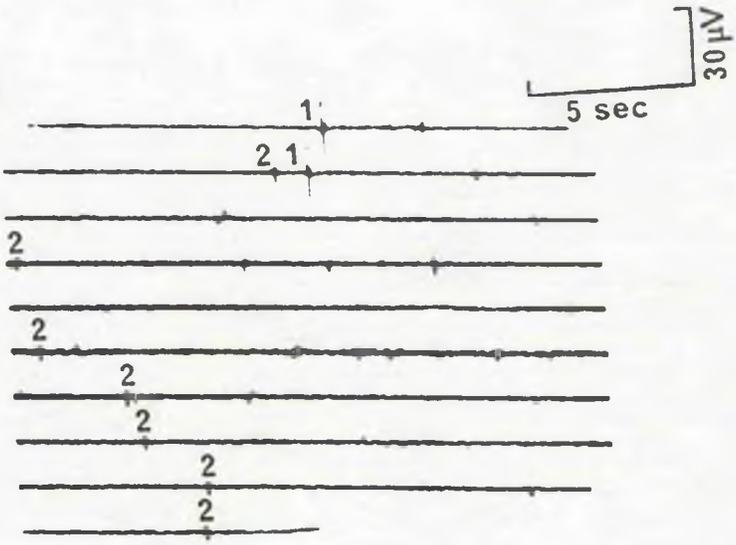
Fig. 1.19. Typical sequence of spontaneous electrical activity occurring in absence of nerve-net activity. Pulse types: (1) SP1, (2) SP2. Two SP1s are seen at the start of the sequence and these are followed by a series of SP2s. Such sequences of activity commonly occur between burst of activity in the nerve-net. The other electrical events in this recording may be regarded as local activity and movement artifacts.

Fig. 1.20. Electrical events associated with spontaneous bursts of activity in the nerve-net. Pulse types: (N) nerve-net pulse, (2) SP2. The nerve-net pulses occur at the beginning of this sequence and are seen to fire at a remarkably regular frequency. Note that the pulses decrease in size as the burst progresses. The nerve-net burst then elicits a sequence of complex electrical activity which is later succeeded by a quiet period lasting about 20 sec. This is followed by a number of SP2s whose firing frequency gradually decreases as the sequence progresses.

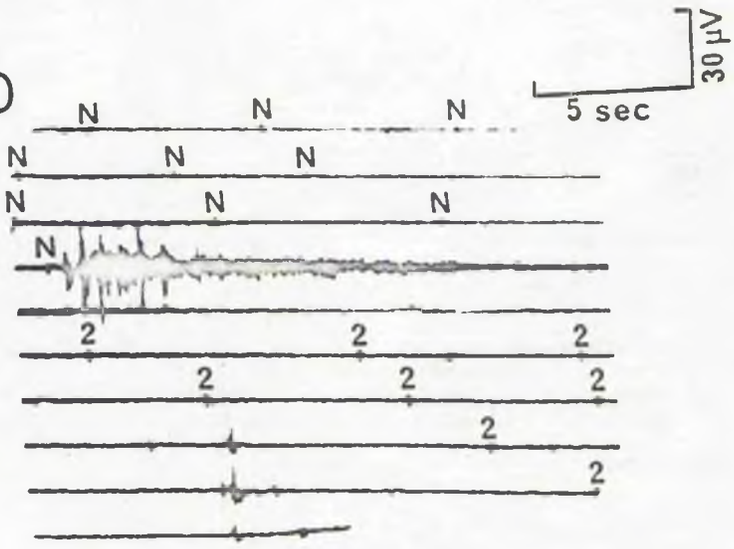
1.18


30 µV
5 sec
L
2
2
1
M
2
2
N

1.19


30 µV
5 sec
1
2 1
2
2
2
2
2
2
2

1.20


30 µV
5 sec
N
N
N
N
N
N
N
N
N
2
2
2
2
2
2
2
2
2

local movements of the electrode, a possible local muscle action potential, a single SP1, a single pulse in the nerve-net, and a number of SP2s. With practice these pulse types may be differentiated from one another by comparing size, shape and duration. Fig. 1.19 shows a typical sequence of electrical activity occurring in the absence of nerve-net activity. There are two SP1s at the beginning of the sequence, which may be evoked by some undetected external stimulus (McFarlane, 1973a). In addition, there is a series of SP2s firing spontaneously at an average frequency of about 1 pulse every 25 sec. Near the end of the sequence the firing frequency is seen to increase slightly. The other events in this recording represent electrode movements unrelated to the activity of the animal.

Monitoring of sequences during spontaneous, high-frequency firing in the nerve-net often proves difficult, for this type of activity sometimes elicits contraction of the tentacles, resulting in detachment of the recording electrodes. On occasions, however, one can maintain the electrodes on a single tentacle for 2-3 hr or more, during which time a very efficient attachment seems to develop. It then proves possible to record the electrical events associated with bursts of nerve-net activity. The sequence shown in Fig. 1.20 is the first time that such a recording has been obtained, and shows, at the beginning, a series of nerve-net pulses firing at a remarkably regular frequency of 1 pulse every 5 to 6 sec. This rhythmical spontaneous firing strongly suggests the involvement of some sort of pacemaker system. Note also that the pulses seem to decrease in size as the sequence progresses. The reason for this is unknown, but one

explanation could be that the pulses evoke small slow contractions in the longitudinal muscles of the tentacles, which would then pull against the suction electrodes, alter the configuration of the recording site, and hence modify the size of the recorded pulses.

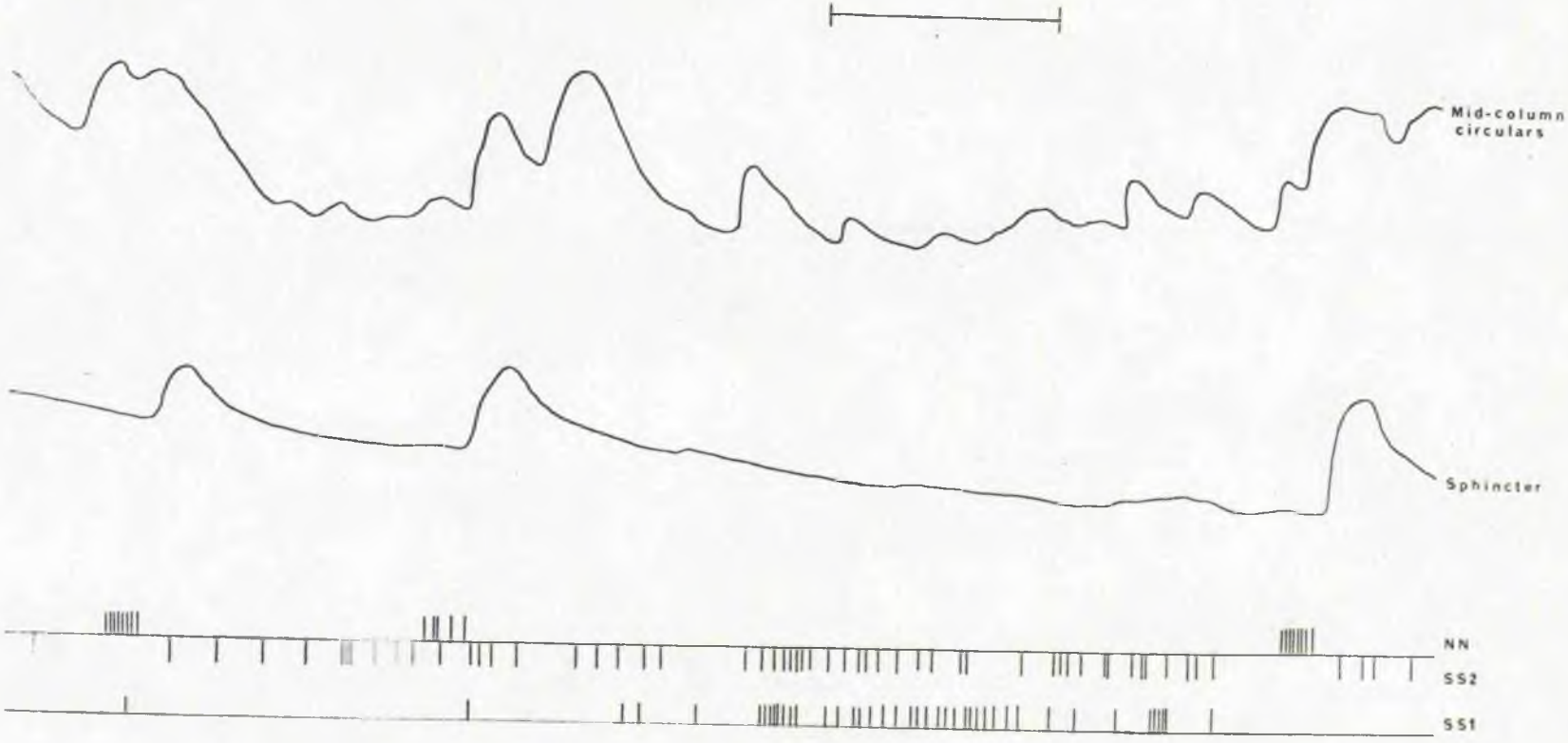
Shortly following this burst of 10 pulses in the nerve-net one can observe a highly complex sequence of electrical activity. This complex activity is often seen at the start of slow contractions in the sphincter or oral disc regions and probably represents local activity in the tentacles themselves. The graded sizes of the larger spikes seen at the start of this activity suggests that these may be local muscle action potentials recorded from the musculature of the tentacles. The smaller spikes may be small muscle action potentials, or they may represent local activity in the tentacular nerve-net. The phenomenon of local activity is discussed at length in Part Two, and will not be dealt with here. The complex activity may be evoked directly, by the preceding burst of pulses in the through-conducting nerve-net, or indirectly, as a result of slow contractions in adjacent muscle groups which would cause the tentacle to pull on the recording electrode and thus trigger compensatory contractions. The latter is probably more likely as complex activity does not invariably accompany a nerve-net burst. In addition, mechanical stimulation, such as stretching, is believed to elicit a burst of nerve-net pulses (Passano & Pantin, 1955). After the complex activity has ceased, there is a quiet period of about 20-25 sec before activity in the SS2 commences. The firing frequency starts at about 1 pulse every 5 sec, but the interval between SP2s gradually increases

as the sequence progresses.

Further experiments employed double preparations to obtain simultaneous recordings of both sphincter and mid-column activity, in order to correlate these with monitored spontaneous electrical events. The movements of the isotonic levers were conveyed to a transducer connected to a conventional pen-recorder. This enables one to record long-term muscular activity without the limitations imposed by smoked-drum recordings. Pulses monitored from the nerve-net, SS1 and SS2 were registered with an event marker. A typical sequence of activity is seen in Fig. 1.21. Note that the SS2 shows rhythmical changes in frequency ranging from about 1 pulse every 8 sec to as low as 1 pulse in 3 min. During low-frequency firing of the SS2 a short, high-frequency burst of activity in the through-conducting nerve-net is seen. These bursts usually consist of about 8-10 pulses at a frequency of upto 1 pulse every 4 sec. This reciprocal relationship between nerve-net and SS2 activity has been noted by McFarlane (1973a) but it is not known whether the two systems have independent pacemakers that are linked in some way, or whether just one system has a pacemaker and this indirectly drives the other. Both the nerve-net and the SS1 show long quiet periods, although Fig. 1.21 shows a sequence occurring between two nerve-net bursts in which the SS1 becomes very active. It is, therefore, possible that some relationship might exist between the nerve-net and SS1, although such sequences are not typical of the majority of recordings. Normally, activity in the SS1 is very irregular and supports the contention that the observed SPIs arise from undetected external stimuli.

Fig. 1.21. Spontaneous activity in a double preparation. Electrical events in the nerve-net (NN), SS1 and SS2 registered on the recording with an event marker. The record confirms that slow contractions of the sphincter occur only after a burst of nerve-net activity. The sphincter does not contract during the burst, indicating that the inhibitory effect is functional during spontaneous activity. The mid-column circulars undergo a number of spontaneous contractions many of which seem to be unrelated to the through-conducted electrical activity. Note that the SS2 shows rhythmical changes in frequency. The nerve-net bursts seem to occur during periods of low-frequency firing in the SS2. The recorded SPIs may arise from undetected external stimuli. Time scale = 5 min.

1.21



The record confirms that spontaneous slow contractions of the sphincter only occur after a burst of activity in the nerve-net. Slow contractions in this muscle are elicited by electrical stimulation at a frequency ranging from 1 shock every 2 sec to 1 every 15 sec (Ross, 1957). The number and frequency of pulses in the spontaneous nerve-net bursts agree with these findings. Note that the sphincter does not contract during the burst, indicating that the inhibitory effect is present during spontaneous activity. This would indicate that inhibition is not a result of some artificial effect induced during electrical stimulation, but assumes a genuine role in the behaviour of the resting animal.

The mid-column circulars differ from the sphincter in showing many spontaneous contractions, even in the absence of through-conducted nerve-net activity. These contractions may be due to myogenic activity in the muscle fibres themselves, or to neurogenic activity in a local nerve-net. The possible mechanisms controlling and coordinating spontaneous activity are discussed at length in Part Two.

The bursts of nerve-net activity seem to induce large, slow contractions in the circulars. Owing to the presence of spontaneous activity it is difficult to estimate precisely when these contractions begin. In some cases, however, the muscle is quiescent enough to allow one to determine the start of slow contractions, and these seem to commence during the nerve-net burst. They often seem to lead the sphincter contraction by upto 1 min, depending on the length of the burst. This offers further evidence that the through-conducted nerve-net activity inhibits slow contraction

of the sphincter until the activity has ceased, and elicits slow contraction of the circulars during the burst. In this respect it is interesting to note that Ewer (1960) found that continuous low-frequency stimulation inhibited spontaneous activity in fresh mid-column rings, but if the preparation had been set up for 3 hr or more this effect disappeared and the rings responded to such stimulation with a slow contraction. The stimulation technique employed was clearly exciting more than one conduction system, and it is possible that the inhibitory effect on the circulars might be due to some action of the SS2 on spontaneous activity. The mechanisms that alter the activity and responsiveness of preparations as they age are not known. They could be related in some way to long-term changes of spontaneous activity in the conduction systems, or to cyclical changes in the excitability of the muscles themselves.

It was noted that even the effects of electrical stimulation may alter with changes in spontaneous activity. If a single electrical stimulus, at an intensity just above nerve-net threshold, is administered during a period of low-frequency spontaneous SS2 activity it often triggers a sequence of about 2-4 nerve net-pulses. If, on the other hand, the same shock is applied during high-frequency SS2 activity such discharges to single stimuli are not elicited. Increasing the intensity also seemsto prevent such repetitive firing. This would suggest that the activity in the SS2 decreases the excitability of the nerve-net in some way. This would offer an explanation for the occurrence of occasional fast contractions during stimulation of the nerve-net alone (see Fig. 1.17B). As yet, however, the means by which SS2 activity can

influence the excitability of the through-conducting nerve-net is unknown.

DISCUSSION

The evidence obtained from this study suggests that every impulse reaching the sphincter by way of the through-conducting nerve-net leaves three effects behind it. These may be classified as facilitatory, excitatory and inhibitory. The facilitating effect, as first described by Pantin (1935a), seems to last for about 2-3 sec at normal temperatures, and prepares the way for fast contraction to a subsequent impulse arriving before facilitation has decayed. The excitatory effect lasts for about 15 sec and contributes to the build-up of the condition which initiates slow contraction (Ross, 1957). The inhibitory effect, as revealed by this investigation, seems to restrain the onset of slow contraction as long as stimulation is maintained, or until the frequency falls below 1 impulse every 16 sec. It does not, however, modify the build-up of slow contraction, but only determines its initiation.

Ross' (1957) analysis of the slow response in the sphincter of Calliactis has been verified. The preparations give smooth, slow contractions in response to frequencies down to about 1 shock every 16 sec. A minimum number of stimuli (usually no less than 6) are required to produce a visible slow contraction, and further stimuli (upto about 30 at a frequency of 1 every 8 sec) serve to increase the size of contraction. Thus, there seems to be an accumulation of discrete excitatory quanta which summate to enhance the slow

contraction. This is in marked contrast to the facilitatory effect set up during the quick response.

As Ross points out, one can only speculate about the mechanisms which make it possible for the sphincter to contract in two ways. The retractors in Metridium senile show both fast and slow contractions, and Batham and Pantin (1954) have argued that if separate fibres were involved, then one would expect buckling to occur during slow contractions, indicating the presence of an unused fast component. This does not happen. Instead, microscopic examination revealed that all the fibres of the muscle sheet are capable of contracting smoothly and slowly. In addition, they could find no histological evidence for the existence of two types of muscle fibre (Batham & Pantin, 1951) and came to the conclusion that the same fibres can contract in both ways.

Robson (1965), in a histological study of the sphincter of Calliactis, makes no mention of different fibre types in this muscle. It is probably fair to assume, therefore, that in the sphincter too the same muscle fibres can contract in both ways. Granted this assumption, the question is raised as to whether the two responses are served by different motor pathways (polyneuronal innervation) in a manner similar to that found in some insects (Pringle, 1939; Hoyle, 1955,1957), or whether they are related to graded changes in muscle membrane potential, as found in crustacean muscle fibres (Hoyle & Wiersma, 1958b). If the former case applies then it may be possible to identify different nerve-endings in the sphincter region with the development of more sophisticated histological and ultrastructural techniques. To verify the latter would require intracellular recordings from the muscle fibres

themselves, and this would prove very difficult with present physiological methods.

Pantin (1965) stated that there was no need to postulate a double motor innervation to explain fast and slow contractions. He believed that facilitated contractions were initiated from all of the neuromuscular junctions, whereas slow contractions were initiated at a few only and were propagated in the muscle. To my mind the very fact that in one case all the neuromuscular junctions are activated, and in the other only a few remain so, implies at least a functional difference between nerve fibres in this region. Ross (1957) also emphasised the essential difference in the nature of the two responses. He points out that fast contractions involve a two-process system requiring separate facilitating and exciting factors, whereas slow contractions seem to involve a straightforward accumulation of excitatory events.

The problem of being unable to obtain direct recordings from the excitable elements themselves prevents one from determining the nature of the inhibitory effect. The fact that slow contraction can be delayed almost indefinitely whilst stimulation is maintained indicates that inhibition is not being confused with latency of the muscle. An active inhibitory effect is clearly evoked by each impulse in the through-conducting nerve-net, and the simplest hypothesis would be to assume that inhibitory fibres are being activated in the nerve-net of the sphincter region. The observation that the inhibitory effect cannot be by-passed by extracellular stimulation of the sphincter region suggests that inhibition is acting at the neuromuscular junction itself. Furthermore, the fact that only the initiation of slow contraction is

inhibited (and not the accumulation of excitatory quanta) would suggest that if conventional synaptic inhibition does occur it presumably does not act presynaptically on the "slow" fibres to cause a decrease in the release of excitatory transmitter.

Further evidence that synaptic inhibition may be occurring is suggested from the graph in Fig. 1.13 which shows that a maximum delay of slow contraction is obtained at a frequency of 1 impulse every 9-10 sec. If one assumes that the duration of this delay is a measure of the relative magnitude of the inhibitory effect, then one could envisage a situation whereby each impulse in an inhibitory fibre releases a quantum of inhibitory transmitter whose production reaches a maximum after about 10 sec. The inhibitory effect then decays as the transmitter is gradually denatured, and disappears entirely after about 16 sec from the arrival of the impulse. Thus, stimulation at intervals of 9-10 sec would cause summation of inhibitory quanta at the peak of production and hence create a maximum inhibitory effect. To carry this speculation further, one must assume that excitatory transmitter from the "slow" fibres has a slower rate of decay. This would mean that once the inhibitory transmitter has effectively been removed there will remain an excess of excitatory quanta whose presence would initiate slow contraction in the absence of further inhibitory stimuli.

The observation that a minimum of about 6 stimuli are required to elicit a slow contraction would imply that the threshold for slow contraction is only reached by a summation of excitatory quanta, and above this level further accumulation of transmitter results in a greater contraction.

The fact that stimulation at a frequency of 1 every 3 sec can evoke slow contraction in the absence of inhibition suggests that the rate of production of excitatory transmitter exceeds that of inhibitory transmitter. This would imply that after 3 sec there is a reversal of the relative excess of transmitter substance, and the inhibitory effect suppresses excitation of the musculature.

It is interesting to note that extracellular electrodes do not record activity associated with slow contractions (McFarlane, & Lawn, 1972). Muscle action potentials, however, are recorded from the sphincter during fast contractions (Josephson, 1966; McFarlane, 1969b). During slow contraction the behaviour of the sphincter may be superficially analogous to that of crustacean muscle fibres. These are relatively inexcitable in that they do not support self-regenerating impulses, and rely entirely on local excitatory junction potentials (e.j.p's) to activate the contractile elements. The multiple innervation of each muscle fibre ensures that the depolarization is distributed throughout its entire length, and thereby achieves synchrony in the shortening of various elements. It is known that the transmitter substance at the crustacean neuromuscular junction is liberated in a quantal fashion (Dudel & Kuffler, 1961), and its effects can be mimicked with glutamate (Takeuki & Takeuki, 1964). It is, therefore, of considerable interest to note that glutamic acid, or a chemically related substance, may have some transmitter function in the supra-oral sphincter of Actinia equina (Carlyle, 1970, 1971, 1972). In this case, however, glutamic acid seems to have a powerful inhibitory effect on electrically induced sphincter contractions.

As far as the inhibitory response in the sphincter of Calliactis is concerned there is not such a close analogy with crustacean muscle fibres. Crustacean muscle does receive an inhibitory nerve supply and the transmitter concerned seems to be gamma-aminobutyric acid (GABA) (Otsuka, Iverson, Hall & Kravitz, 1966). Stimulation of the inhibitory nerve can cause relaxation of the muscle, regardless of any excitatory input (Fatt & Katz, 1953; Hoyle & Wiersma, 1958a). This effect could not be demonstrated for Calliactis sphincter and suggests that the analogy should not be extended too far.

Ross (1960b), in a study of the effects of ions and drugs on sphincter preparations of Calliactis, could discover no drug (GABA included) capable of producing an inhibitory effect, although glutamic acid was not tested. Most drugs did not greatly affect either quick or slow contractions - only tyramine, tryptamine and adrenaline gave promising results. Adrenaline had no effect on the quick facilitated response, but was the only drug to cause direct slow contractions and also enhance these during electrical stimulation. Unfortunately, this sensitivity to adrenaline could not be modified by adrenaline inhibitors, adrenergic blocking agents, or the potentiating drug cocaine. This would suggest that neuromuscular transmission in the slow response, despite the effects of adrenaline, is not an adrenergic mechanism. Ross was led to the opinion that a complementary relationship existed between the two responses "as if some transmitter, produced when impulses reach the muscle, contributes something to any quick contractions that occur, and the remainder (or its products) evokes the slow contraction". The observation that the fast contraction cannot be inhibited during low-

frequency stimulation would imply that a single transmitter is not involved in both quick and slow responses; although if the properties of the transmitter could change within 3 sec of release such a suggestion might be plausible. Clearly, the question of neuromuscular transmission in actinians remains something of an enigma, and until we have a more intimate understanding of these processes one can do little more than guess at the mechanisms involved.

Propagation of fast contractions of the sphincter appears similar to that found in vertebrate muscle fibres. Activation of the contractile elements seems to be achieved by a uniform self-reinforcing impulse which rapidly spreads throughout the muscle fibre and thereby synchronizes the shortening of its component parts. It must be stressed, however, that these ideas on the nature of excitatory and inhibitory mechanisms are speculations based on the evidence at hand, and are offered only as a possible explanation for the effects observed. The fact that the time-scales are greatly extended in the responses found in sea anemones compared with those encountered in higher groups constitutes the major functional difference between them.

The innervation of the sphincter of Calliactis has been described by Robson (1965). Processes from the nerve-net above the circular muscle, or from the retractor nerve-net of a mesentery, seem to pass directly through the mesogloea to link with the sphincter muscle beneath. Unfortunately, nerve terminations on actual muscle fibres have not been seen, but it seems likely that these must exist. The muscle fibres themselves line the periphery of hollow tubes arranged in groups within the mesogloea, and

thus correspond with other actinian muscle fibres in that each is attached to the mesogloea along one surface only. Most of these fibres have no connection with the epithelium and their nuclei are seen within the tubes. The muscle also has its own supply of bipolar nerve cells which probably run within the muscle tubes. Robson mentions that some of the nerve-net supplying the sphincter runs over the circular muscle, aand this may have some bearing on the observation that the circulars show slow contractions during stimulation of the sphincter. This would add strength to the conjecture that inhibition occurs only at the neuromuscular junction of the sphincter and not presynaptically, for this would presumably cause inhibition of the circulars too. It is, perhaps, unfortunate that present histological techniques do not reveal any major differences among the nerve cells supplying the sphincter, as this would provide some morphological support for the physiological findings.

In studying sea anemones one is left with this remarkable paradox between their apparent functional and behavioural complexity on the one hand, and the seemingly simple structural organization of their nervous systemson the other. As Ross (1967a) has pointed out "the most careful work has not revealed local concentrations of nerve cells, or centres, or pathways of any kind, which would provide structural counterparts for the functional capacities of the system". One can only come to the conclusion that actinian nervous systems may appear superficially primitive, but they are certainly not simple.

The final question that remains unanswered concerns the role of sphincter inhibition in the general behaviour of

Calliactis. At this stage, however, the significance of the inhibitory effect can only be surmised. It is unlikely that C. parasitica from the English Channel would encounter external mechanical stimulation capable of reproducing the regular low-frequency firing of the nerve-net required to induce sphincter inhibition. As mentioned previously, Pagurus bernhardus, the commensal hermit crab partner of C. parasitica, does not play an active part in establishing the association (Ross, 1960c, Ross & Sutton, 1961a). Furthermore, in those species of Calliactis that are prodded and stroked by their commensal pagurids in order to facilitate detachment, the induced state of relaxation would appear to be a side-effect of this type of stimulation, rather than a necessary prelude to detachment. Ross & Sutton (1970) mention that tapping the column of C. tricolor for 2-4 min could bring about detachment, even though the anemone sometimes remained completely closed during stimulation. They also noted that the crab Dardanus venosus often detached C. tricolor in a closed condition. Obviously, the detachment process in Calliactis can occur in the absence of the inhibitory effect, and this agrees with McFarlane's (1969c) findings that detachment is coordinated by the SSl. It would appear, therefore, that the manipulations of the crab evoke detachment of the pedal disc by exciting the SSl, and the accompanying state of relaxation is an incidental effect, perhaps brought about by some form of sensory adaptation, or inhibitory stimulation of conduction systems.

Since the inhibitory effect does not seem to be a vital prerequisite for "shell climbing", it is suggested here that the major roleoof sphincter inhibition in Calliactis is

to control and coordinate slow closing movements of the anemone. Needler & Ross (1958) have suggested that such closures are employed to expel water from the coelenteron from time to time. Fresh water is then taken in, perhaps by ciliary action, to replenish the supply. Such pumping and circulating activities occur periodically, and it is possible that these sequences are elicited by spontaneous activity in the through-conducting nerve-net. Evidence has been presented in this study to suggest that periodic bursts of activity in the nerve-net occur spontaneously, and the frequency and number of pulses in these bursts seem to fall within the range of stimulation known to induce sphincter inhibition. Once the inhibitory effect from such a burst has died away, slow contraction of the sphincter may ensue, as shown in Fig. 1.21.

It is obviously desirable that the sphincter should not begin to contract before theoral disc has been pulled down by the retractor muscles, and themmajor role of the inhibitory effect may be to hold off this sphincter contraction. One could envisage the slow closure mechanism occurring as follows:

1) A spontaneous burst of activity in the through-conducting nerve-net is elicited, perhaps by some sort of pacemaker system that may be linked with SS2 activity in some way (McFarlane, 1973a). Alternatively, the burst may be evoked by some sort of chemoreceptor (or perhaps the nerve-net itself) responding to changes in the nature of the coelenteric fluid when it needs replenishing.

2) The impulses in the nerve-net evoke slow contractions of the longitudinal m senteric muscles, which pull the oral disc downwards. Ross & Sutton (1970) mention that electrical

stimulation at frequencies below 1 pulse per sec produces such an effect in C. polypus after about 10-20 stimuli. It is probable that the oral disc radial and tentacle longitudinal muscles would also respond to thennerve-net burst with slow contractions, and these would accompany the retraction of the oral disc. Slow contraction of the oral disc radials during low-frequency stimulation of the nerve-net has been noted in Tealia felina (see Part Two, and McFarlane & Lawn, 1972).

3) Once the oral disc has been withdrawn in this way, and the nerve-net burst has ceased, the sphincter is then released from inhibition andmmay contract without fear of constricting the oral disc or tentacles in any way.

If such sequences were coordinated merely by variations in latency of the different muscles concerned the slow closure would have to proceed as a stereotyped series of reflexes, in a similar manner to the protective withdrawal response, which is presumably a highly specialized mechanism (designed to prevent damage to the delicate oral disc and tentacles during strong stimulation) involving fast contractions alone. The fact that slow contraction of the sphincter can be held off for as long as low-frequency nerve-net activity continues, implies that a certain degree of variation and flexibility may be introduced into the slow sequences. For example, if a large volume of water, or perhaps a mass of food, needs to be expelled from the coelenteron, the retraction sequence and the subsequent decrease in volume may be prolonged and accentuated simply by a continuation of nerve-net activity. The accompanying inhibitory effect would overcome the problem of the sphincter contracting and preventing expulsion of such material from the mouth.

Should this conjecture prove true then it would appear that the inhibitory effect introduces an additional element of variability into the behavioural repertoire of sea anemones. The possibility that inhibition may be involved in such basic behavioural activity as slow closing movements would suggest that inhibitory mechanisms might exist in a wide variety of sea anemones, and this should be looked for in future studies. The discovery of such a fundamental mechanism in a muscle studied as thoroughly as the sphincter of Calliactis suggests that we should, perhaps, revise some of our concepts concerning the means by which sea anemones control and co-ordinate their considerable range of behavioural activities.

SUMMARY OF PART ONE.

1. The responses of the sphincter muscle of *Calliactis parasitica* to mechanical and electrical stimulation have been investigated. The results confirm the presence of a quick facilitated response and a slow accumulated response. An additional response has been discovered which inhibits the initiation of slow contractions.

2. This inhibitory response is active at frequencies ranging from 1 pulse every 4 sec to 1 every 16 sec, and has a maximum effect at a pulse interval of 9-10 sec.

3. Slow contraction of the sphincter can be delayed for periods of upto 30 min or more, providing inhibitory stimulation is maintained, whereas slow contraction of the column circulars is usually evoked during the period of stimulation.

4. Fast contraction of the sphincter can be elicited during inhibitory stimulation and appears to be totally independent of this effect.

5. The inhibitory response is mediated by the through-conducting nerve-net, and no other conduction system seems to be directly involved.

6. A study of spontaneous activity in unstimulated preparations has revealed the means by which activity in the conduction systems can control contractions of the sphincter and column circulars. Evidence is presented to suggest that inhibitory responses play an active role in these functions.

7. The possible nature of the mechanisms involved in the control and coordination of sphincter activity is discussed in the light of these observations. The nervous system seems to possess a functional complexity which belies its primitive structural organization. It is suggested that sphincter

inhibition plays an important role in basic behavioural activity, such as slow closure sequences.

PART TWO

EXPANSION AND CONTRACTION OF THE ORAL DISC IN TEALIA FELINA

INTRODUCTION

The feeding behaviour of sea anemones may be broadly separated into two phases: the feeding response and the pre-feeding response. The feeding response involves capture and ingestion of solid food. In this case, contact of tentacles or oral disc with the food results in a discharge of cnidae which cause the food material to adhere to the tentacles. This is then followed by a series of ingestive movements, in which the food is transferred to the mouth via the tentacles and oral disc. Stephenson (1928) noted that food transference may involve both ciliary and muscular activity, as in Metridium. In certain large anemones that are accustomed to large food masses (e.g. Tealia, Bolocera, Actinostola) muscular activity alone is responsible for food transfer. Once the food has been conveyed from the periphery to the centre of the oral disc the mouth opens to permit the food to enter the coelenteron, where it may be digested over a period of several hours, or even days. Pantin & Pantin (1943) have shown that the feeding response is normally elicited by a combination of mechanical and chemical stimuli.

This investigation, however, is mainly concerned with activity encountered in the pre-feeding response. This activity involves expansion of the oral disc and extension of the tentacles in response to dissolved food substances. Expansion is sometimes accompanied by slow bending movements

and extension of the column, which often cause the anemone to "sway" very slowly from side to side. This behaviour obviously increases the chance of contacting nearby food by extending the food-capturing range of the tentacles and oral disc.

The ability of the sea anemone to assume an active role in increasing the chances of food capture has been often noticed in the past, and is obviously an important component of feeding behaviour in these animals. Tentacle extension and expansion have been recorded in some of the very earliest accounts of actinian behaviour. The Roman naturalist Pliny records in his "Natural History" (translated by Bostock & Riley, 1855) that the "sea nettle" (sea anemone) will "spread out its branches" in order to "sieze and devour" its prey. In the 18th century, John Ellis (1767) mentions that the "Actinia or Animal Flowers .... found all around the coasts of England" are capable of "extending their bodies and claws (tentacles) in search of food". The great 19th century naturalist P.H. Gosse (1860), in his masterly account of the British sea-anemones and corals, does not specifically mention preparatory feeding activity, but quotes some of his correspondents who have observed tentacle extension in several specimens of Sagartia.

The first authoratative account of the pre-feeding response is given by Pollock (1883) who observed that the placing of any kind of food near to a closed anemone (in this case, the "common" sea anemone) would induce it to open. This response could be obtained under field or laboratory conditions. Later accounts of preparatory feeding activity refer mainly to Metridium senile (Allabach, 1905; Parker, 1919; Pantin, 1950), where the response to dissolved food substances

involves expansion of the oral disc followed by extension and "swaying" of the column. Pantin notes that although these activities are not connected with the actual ingestion of solid food, they are clearly purposive in character. None of these studies revealed the nature of the mechanisms involved in the control and coordination of the pre-feeding response. The through-conducting nerve-net is unlikely to mediate the expansion of the oral disc, since stimulation of the nerve-net elicits only fast and slow contractions in the muscles concerned (Batham & Pantin, 1954).

The recent discovery of multiple conduction systems in sea anemones (McFarlane, 1969b) suggested that much of the behavioural physiology of these animals should be re-investigated. The ectodermal slow system (SS1) has been shown to control detachment of the pedal disc during shell-climbing behaviour in Calliactis parasitica (McFarlane, 1969c). Recent work by McFarlane (1970) has shown that the SS1 in Tealia felina is somehow involved in the pre-feeding response. Although many of the previous studies on preparatory feeding activity have been concerned with Metridium senile, this species proved unsuitable for electrophysiological work, as the animal would often retract and detach the recording electrodes. Tealia felina was found to be much less irritable, permitting continual monitoring of electrical activity for several hours.

It was found that dissolved food substances contacting the column of Tealia elicit activity in the SS1, followed by expansion of the oral disc and lowering of its margin. This expansion response could also be elicited by electrical stimulation of the SS1.

The present study is concerned with the nature of the mechanisms involved in the control and coordination of oral disc activity. The effect of the SSl on the radial muscles of the oral disc has been examined, and it appears that oral disc expansion can be explained, in part at least, by an observed inhibition of the radial muscles. In the light of this investigation these muscles may now be regarded as having a dual control; excitatory from the nerve-net, and inhibitory from the SSl. This work is of additional interest, for if the SSl proves to be a genuine neuroid conduction system (see review by Mackie, 1970) then this is the first account of muscular inhibition by such a system.

MATERIALS AND METHODS

Specimens of Tealia felina var. lofotensis were obtained from the North Sea near St. Andrews. Before being used in experiments they were kept in the aquarium at the Gatty Marine Laboratory for several months to allow them to recover from the effects caused by dredging.

Only specimens in good condition with expanded oral disc diameters of 6-12 cm were used. These were starved for three days before use to ensure that feeding activity would not modify the results of the experiments.

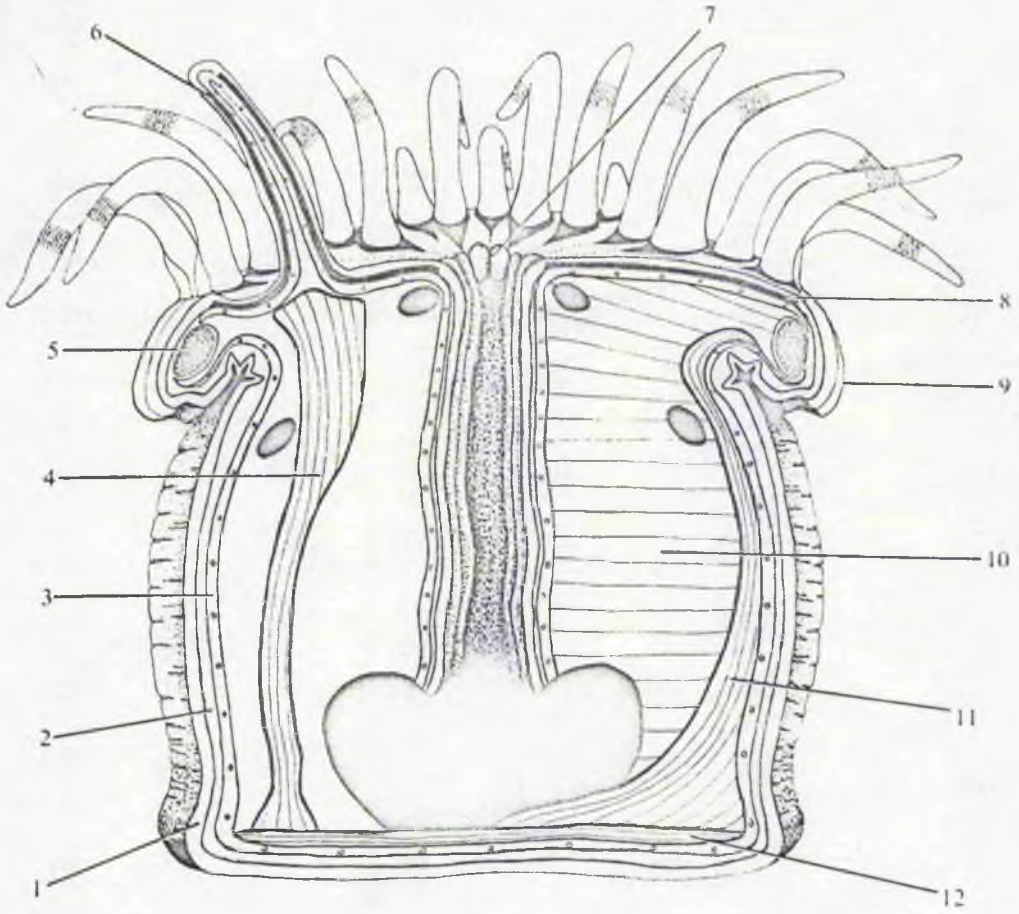
The radial muscles of the oral disc were chosen for this investigation as they appear to play a vital role in the expansion response. The position of the radials and the general arrangement of the muscles in Tealia are shown in Fig. 2.1.

The radials are strongly developed, in contrast to the sparse endodermal circular muscles of the oral disc. Both

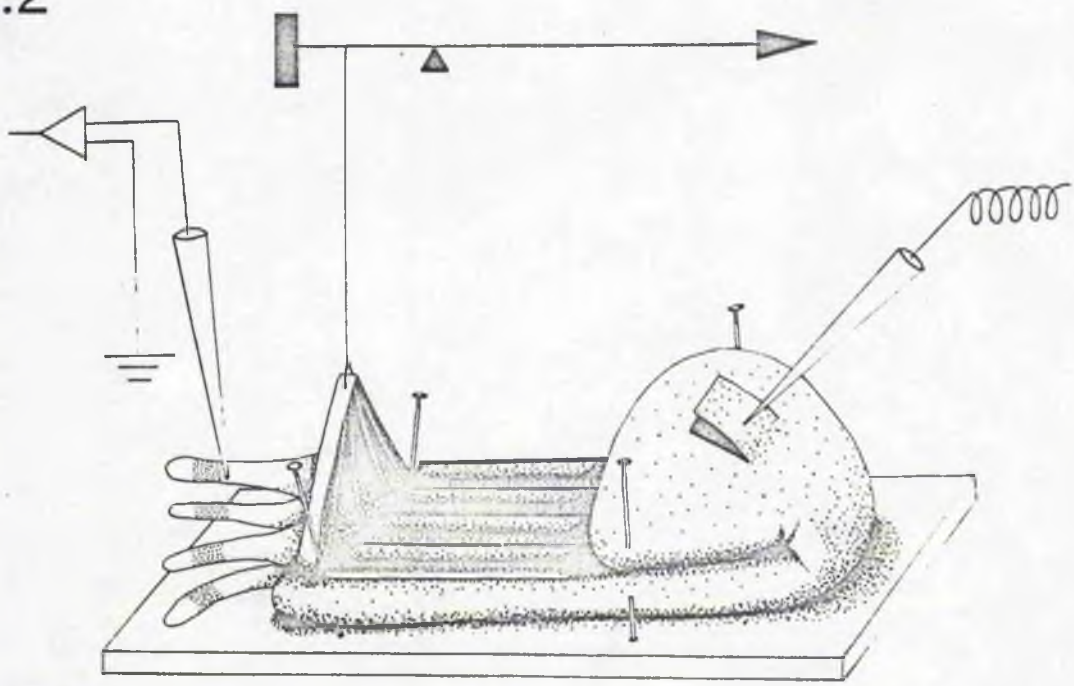
Fig. 2.1. Muscular anatomy of Tealia felina var. lofotensis. The diagram shows on the left the structure of the endocoelic face of a non-directive perfect mesentery, and on the right the exocoelic face. 1, Ectoderm; 2, mesogloea; 3, endoderm (includes circular muscle layer); 4, retractor muscle; 5, sphincter muscle; 6, tentacle ectodermal longitudinal muscle; 7, siphonoglyph; 8, ectodermal radial muscle of oral disc; 9, collar; 10, transverse muscles; 11, parieto-basilar muscle; 12, basilar muscle. Based in part on description by Stephenson (1928).

Fig. 2.2. The radial muscle preparation. Suction electrodes were used for recording and stimulation. The recording electrode is attached to a tentacle. The stimulating electrode is shown here attached to a flap in order to stimulate the SS1 alone. The flap technique was used to stimulate different conduction systems, as described in Part One.

2.1



2.2



the radials and the tentacle longitudinals are ectodermal in origin, although in all but the smallest specimens they may lie totally or partly enclosed by mesogloea (Carlgren, 1921). These ectodermal muscle fibres are the products of modified epithelial cells, and differ from their endodermal counterparts in that they are separate from the epithelial supporting cells above them; i.e. they do not form a musculo-epithelium (see Stephenson, 1928). Other muscles referred to in this study are the transverse mesenteries, the retractors, and the sphincter.

The preparation used in most experiments was obtained in the following way. An anemone was bisected longitudinally and a thread was sewn into one of the pair of siphonoglyphs that lie at the pharyngeal edge of the oral disc. Attachment was made here as this structure was found to be less liable to tearing than other regions of the disc. The preparation was then trimmed to a sector approximately equal to 45^{o} of the oral disc, and the pedal disc was removed. Generally, the mesenteries were excised to within a few millimetres of their attachment to the column wall and oral disc. It was found that this did not destroy the pathway of the through-conduction system. A shallow flap comprised of ectoderm and superficial mesogloea was cut near the base of the column. The basal part of the column curls over on itself when the preparation recovers, hence allowing the stimulating electrode to be attached to the flap. The conduction systems capable of stimulation by the flap technique have been described in Part One.

Preparations were pinned with the ectodermal surface of the column facing downwards. They seemed to recover more

quickly and were generally more active if anaesthetic were not used during the operations. All were left to recover in running, well-oxygenated sea water for 16-24 hours, and during experiments were retained under similar conditions at temperatures ranging from 7-12°C. Most studies were made between 16 and 48 hr after operation and preparations more than 72 hr old were discarded.

Activity of the radial muscles was recorded with a light isotonic lever writing on a kymograph. Attached preparations were found to relax to about one-half of their normal expanded length. Suction electrodes were used for both recording and stimulating (Josephson, 1966). The experimental arrangement is summarized in Fig. 2.2. The recording electrode is shown attached to the mid-region of a tentacle in order to monitor the results of electrical stimulation. This is necessary to ensure that the desired conduction system is being stimulated. Pulses from the recording electrode were amplified and displayed as described in Part One. All electrical stimuli were of 1 msec duration.

RESULTS

Electrical Activity

Electrical activity in Tealia felina seems identical to that encountered in Calliactis parasitica, as described in Part One. One fairly consistent observation is that the recorded pulses in Tealia, especially in the SS1, often tend tonbe larger in amplitude than those in Calliactis. McFarlane (1970) quoted a maximum figure of 60μV for the SP1 in Tealia, although he found the normal range extended from 20-30μV. It

was noted during the present study, however, that much wider ranges and greater amplitudes are encountered whilst recording from certain specimens of Tealia. The largest amplitude obtained for an SP1 during these observations was found to be 112μV (see Fig. 2.3) but it should be stressed that this is an abnormally large value, even for T. felina. The recorded pulse shown in Fig. 2.3 exhibits the biphasic form typical of SS1 pulses. The pulse duration is approximately 250 msec. These factors of amplitude, shape and duration may be used by the observer to distinguish the SP1s from other electrical activity (see Part One).

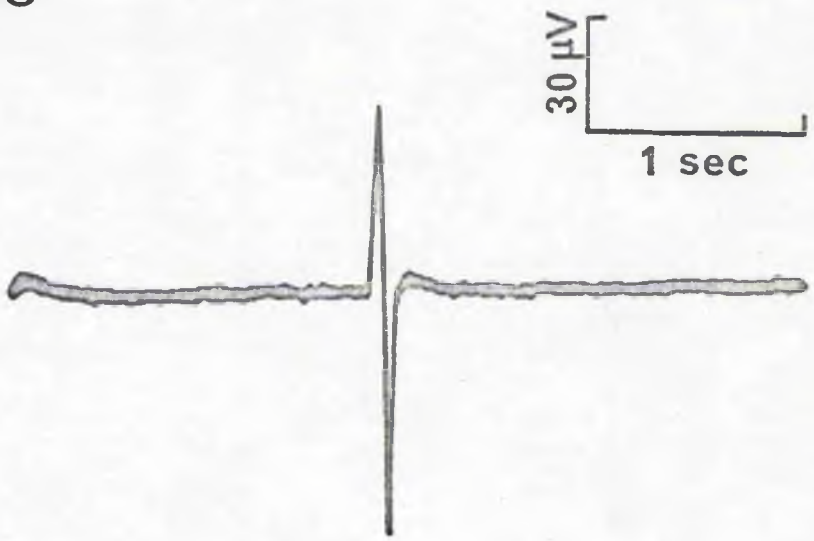
Pulses recorded from preparations seem identical to those recorded from the intact animal. Monitoring of SP1s may become difficult when the SS1 is being repeatedly stimulated at relatively high frequencies. Repetitive stimulation at frequencies of 1 shock every 3 sec down to 1 shock every 15 sec results in a progressive increase in response delay and a decrease in SP1 amplitude. This effect at frequencies of 1 every 4 sec and 1 every 6 sec is shown in Fig. 2.4.

At stimulation frequencies greater than 1 shock every 3 sec conduction in the SS1 rapidly fails. At these frequencies the SP1 may become too small to distinguish, and it proves difficult to tell whether the system has ceased to conduct, or whether the pulse has become too small to be visible above the noise level. Even at lower stimulation frequencies the SS1 occasionally failed to respond to some of the shocks in a stimulus series. These effects of repetitive stimulation are also obtained with Calliactis parasitica (McFarlane, 1969b). In the light of these observations it is interesting to note that intracellular

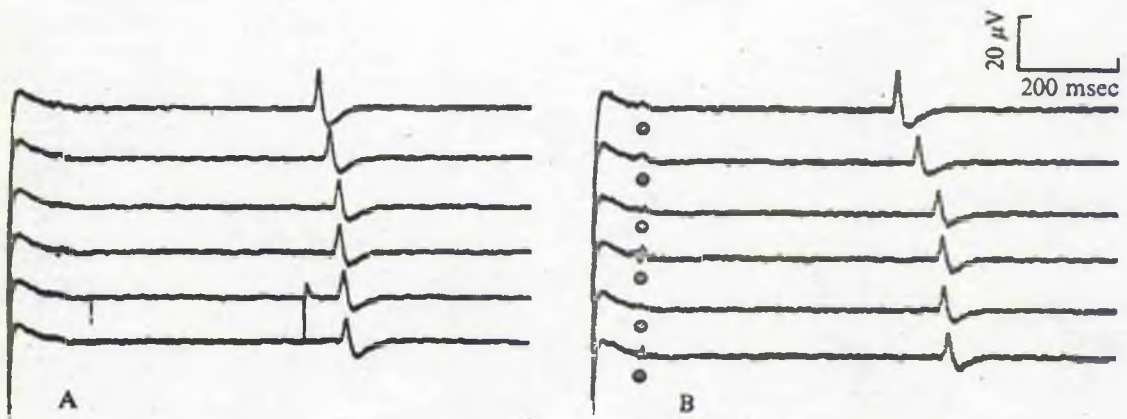
Fig. 2.3. Recording of a large-amplitude SP1 in Tealia felina. Recording electrode on a tentacle, stimulating electrode attached to a flap. The biphasic waveform is typical of SP1s, although shape and duration of these pulses may vary considerably between different animals, or even in the same animal at different times. The amplitude of this particular pulse (112μV) is abnormally large for an SP1. The usual amplitude for an SP1 in Tealia varies between 20-30μV.

Fig. 2.4. The effect of repetitive stimulation of the SS1. Recording electrode on a tentacle, stimulating electrode on intact column. Stimulus frequencies: (A) 1 shock every 6 sec, (B) 1 shock every 4 sec. Responses shown to six shocks, reading from top to bottom. Note increase in response delay of SP1 and also decrease in pulse size, both changes being more marked at the higher stimulus frequency. Pulse associated with the nerve-net is just detectable in B (position shown by dots). There is no obvious change in response delay for this pulse. Note the considerable difference in response delay between the nerve-net pulse and the SP1.

2.3



2.4



recordings of the pulse associated with myoid conduction in the siphonophore Nanomia bijuga reveal that repolarization after activity may take upto 3 sec (Spencer, 1971).

Responses to Electrical Stimulation

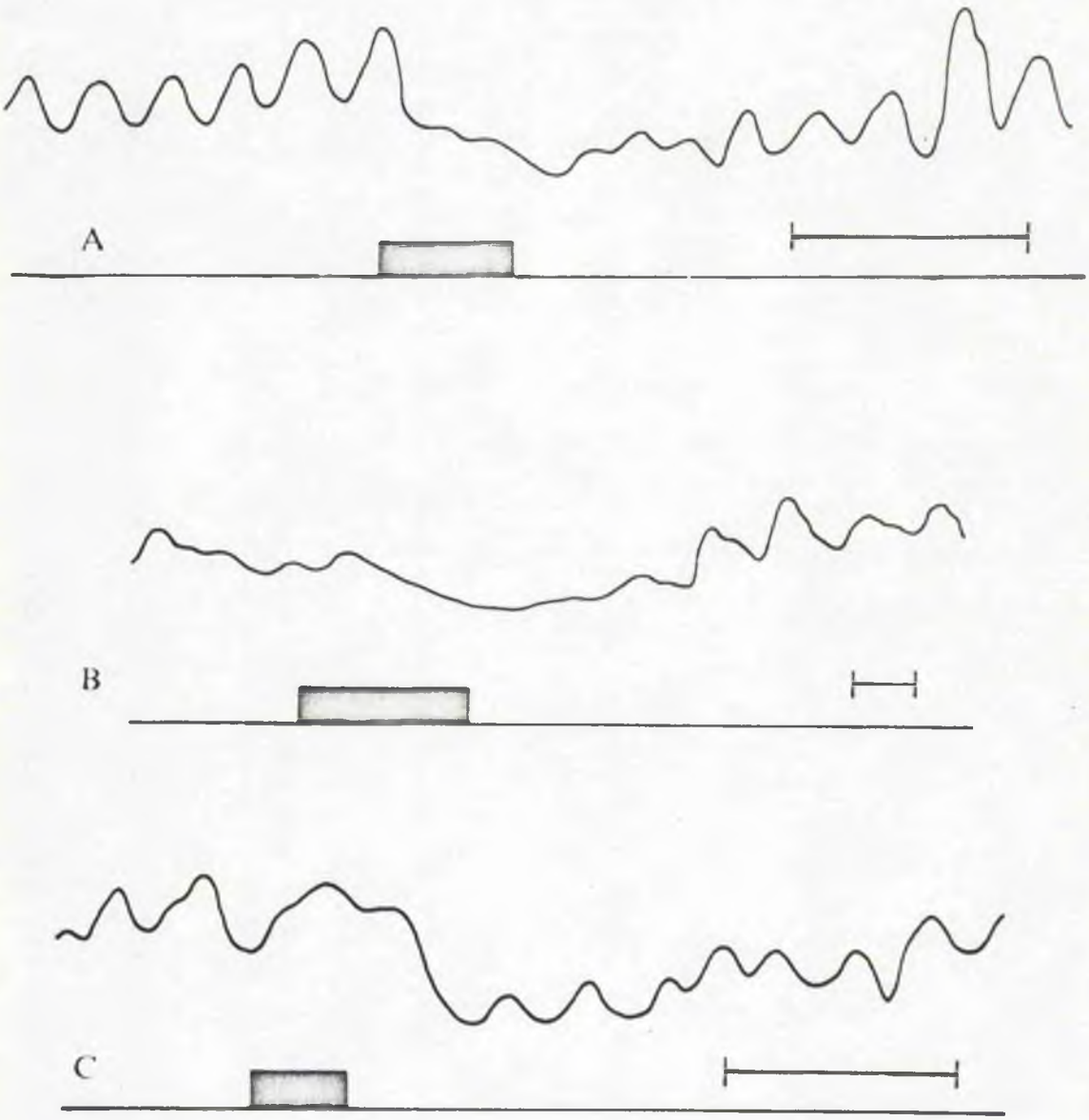
The majority of oral disc preparations show irregular spontaneous contractions. Fig. 2.5 shows the action of the SSl on spontaneously active preparations. Note that the SSl at these frequencies causes a reduction in the size of the spontaneous contractions, a lengthening of the radials, and often changes the original frequency of the spontaneous contractionsduring recovery.

Fig. 2.5A shows the response to 35 shocks at a frequency of 1 every 5 sec. A clear response is visible within 1 minute of the start of stimulation, i.e. after approximately 10 shocks. A consistent aspect of the response is that the inhibitory effect persists beyond the end of stimulation and the recovery process involves a gradual increase in spontaneous activity and decrease in length of the radials. Contractions occurring late in the recovery period are often larger than normal spontaneous contractions.

Fig. 2.5B shows the effect of 30 shocks at a frequency of 1 shock every 30 sec. Inhibition of spontaneous activity accompanied by a lengthening of the radials is again seen, even at these low frequencies. Rarely, relaxation was not obvious during stimulation, but became obvious shortly afterwards. This is shown in Fig. 2.5C in which 20 shocks were applied at 1 every 6.3 sec. Note, however, that an inhibitory effect acts on the spontaneous contractions during stimulation, even though lengthening occurs only after stim-

Fig. 2.5. Action of SSl stimulation on spontaneously active preparations. Stimulating electrode on a flap so that only the SSl is stimulated. (A) Response to 35 shocks at 1 every 5 sec, (B) 30 shocks at 1 every 30 sec, (C) 20 shocks at 1 every 6.3 sec. In this and all subsequent kymograph records contraction is upwards. Note reduction in spontaneous activity, increase in length and the prolonged recovery phase. There is a delayed onset of relaxation in C. Time scale = 5 min.

2.5



ultation has ceased.

It seems that stimulation at these frequencies causes the SS1 to act on the relaxation phase of the spontaneous contractions. SS1 activity seems to cause a prolongation of this phase by inhibiting the next contraction in the series and hence evoking a lengthening of the radials. This is shown very markedly in Fig. 2.6, in which 20 shocks were applied at a frequency of 1 every 8 sec. Note that once a spontaneous contraction has started the SS1 does not seem to modify or inhibit it early in the stimulation series (about 4-5 SPIs). After about 5-10 SPIs, however, the next contraction in the series appears to be completely inhibited, hence resulting in a prolonged relaxation phase and subsequent lengthening of the radials. The recovery phase takes about 10 min from the start of relaxation, and again involves a gradual decrease in length and increase in spontaneous activity. Note also the change in frequency of the spontaneous contractions before and after stimulation.

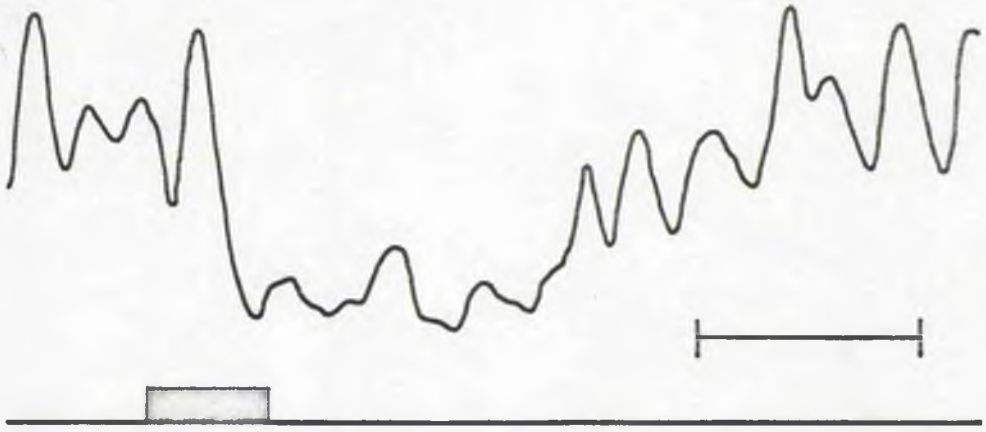
Stimulation of the SS1 at relatively high frequencies proves difficult to monitor owing to the fatigue effects of repetitive stimulation (see Fig. 2.4). In some preparations, however, it was possible to stimulate the SS1 at frequencies of 1 shock every 2 sec and elicit all but 2 or 3 SPIs in a series of 30. Stimulation at this frequency gives the response shown in Fig. 2.7A, in which there is a dramatic and rapid relaxation effect following stimulation. The recovery of spontaneous activity after inhibition is likewise relatively rapid, although the time taken for the radials to assume their previous length is about 10 min from the start of relaxation.

Stimulation of the SS1 at very low frequencies

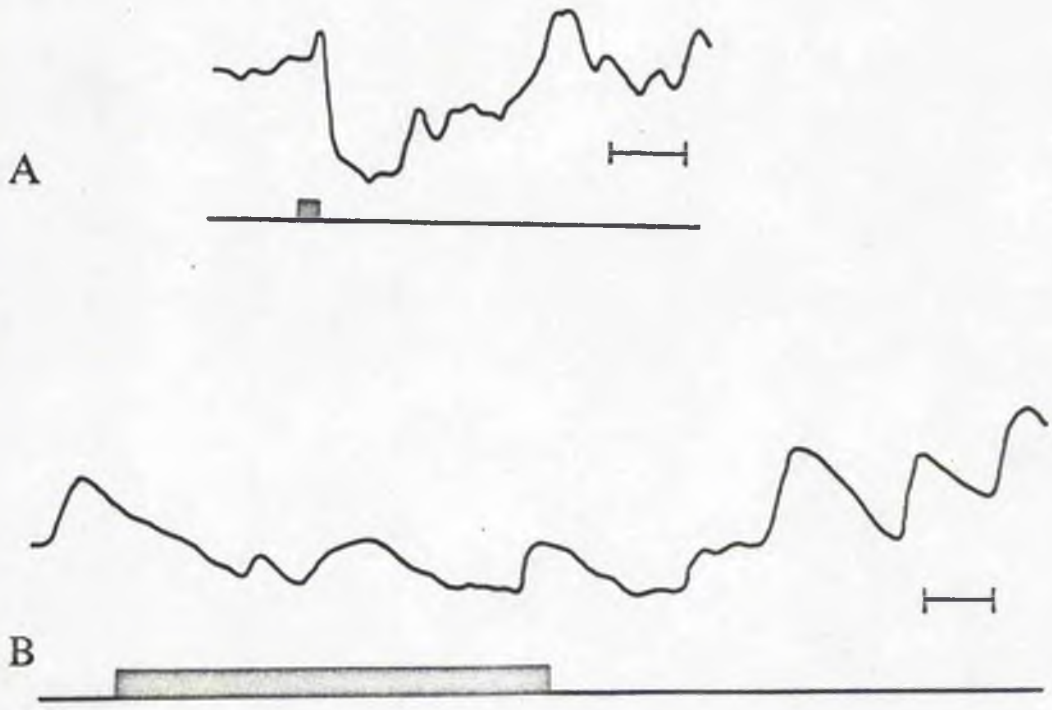
Fig. 2.6. Inhibition of spontaneous contractions by stimulation of the SS1. The stimulating electrode was attached to a flap, and a series of 20 shocks at 1 every 8 sec was administered. The spontaneous contraction occurring during stimulation seems to be relatively unaffected. The next contraction in the series, however, appears to be inhibited, thereby causing a prolonged relaxation phase and subsequent lengthening of the radials. Note the prolonged recovery phase and the change in frequency of the spontaneous contractions before and after SS1 stimulation. Time scale = 5 min.

Fig. 2.7. Response of preparation to stimulation of the SS1 at high and low frequencies. (A) 30 shocks at 1 every 2 sec, (B) 30 shocks at 1 every 60 sec. In A there is a relatively rapid inhibitory response followed by an equally rapid recovery phase. In B the inhibitory response is difficult to detect although the recovery phase is quite distinctive. Time scale = 5 min.

2.6



2.7



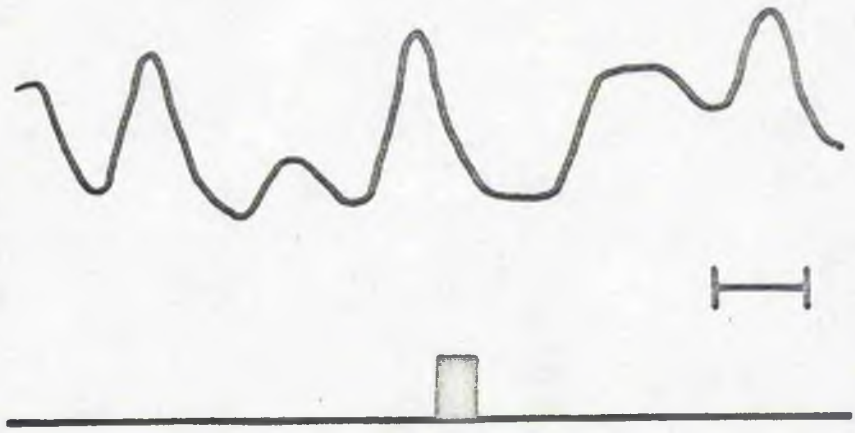
produces less noticeable variations in radial activity. Fig. 2.7B shows the effect of 30 shocks at a frequency of 1 every 60 sec. If one compares this with the effects of stimulating at a frequency of 1 shock every 30 sec for the same number of shocks (as depicted in Fig. 2.5B) one notices that the inhibitory and lengthening effects become increasingly difficult to observe as the stimulation frequency is lowered. It is, however, possible to detect a slight lengthening and a decrease in spontaneous activity in Fig. 2.7B. There is also a marked recovery process following this inhibitory effect.

From these observations it appears that the minimum frequency required to produce a clear relaxation is approximately 1 shock every 60 sec, and the maximum frequency is 1 shock every 2 sec. It is difficult to determine the optimum frequency of stimulation, since for a given number of shocks at high frequencies a rapid relaxation is obtained but is maintained for only a short period, whereas the converse is true for the same number of shocks at low frequencies. Fig. 2.7B also indicates that continued stimulation inhibits the recovery process of the radials; an effect which parallels the inhibition of slow contraction in the sphincter of Calliactis parasitica (see Part One).

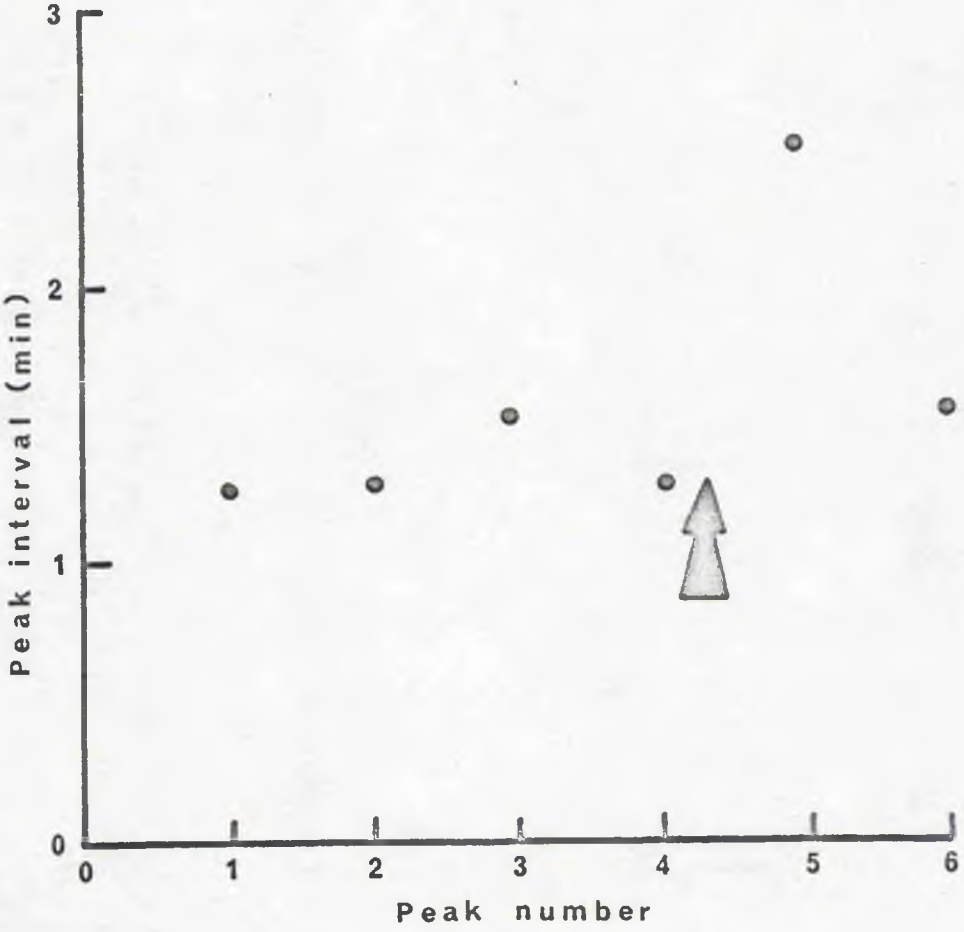
It is difficult to state with certainty the minimum number of stimuli, at a given frequency, required to cause an inhibitory effect. This is owing to the presence of spontaneous activity during stimulation, for once a spontaneous contraction has commenced during a stimulation series it will complete itself before any inhibitory and lengthening effects may be observed (see Fig. 2.6). In some

Fig. 2.8. Minimum number of SPIs required to evoke an inhibitory effect. (A) Response to 5 shocks at 1 every 5 sec. Although no lengthening of the radials can be observed, the contraction following stimulation appears to be slightly delayed. Time scale = 1 min.
(B) Graph denoting interval between contraction peaks in Fig. 2.8A. The 5 SPIs were administered at the point shown by the arrow. The graph depicts a significant delay in the onset of the spontaneous contraction which immediately follows the applied stimulus.

2.8



A



B

cases, however, it would appear that a slight inhibitory effect occurs with as few as 5 shocks at a frequency of 1 every 5 sec. This is shown in Fig. 2.8A which indicates that although there is no overall lengthening of the radials, the contraction which follows stimulation seems to have been slightly delayed by the preceding pulses. This is perhaps indicated more clearly if one plots the interval between contraction peaks against peak number, as shown on the graph in Fig. 2.8B. It can be seen that a relatively large increase in peak interval occurs as the result of applying 5 shocks to the preparation. This may be interpreted as the minimum number of shocks (at a frequency of 1 every 5 sec) that will produce a visible inhibitory effect. The fact that a lengthening of the radials does not appear to occur in this case again suggests that the SS1 initially inhibits the spontaneous contractions. In Fig. 2.8A it would appear that 5 pulses are insufficient to cause a prolonged relaxation phase of the spontaneous contraction, thus preventing the lengthening effect from occurring.

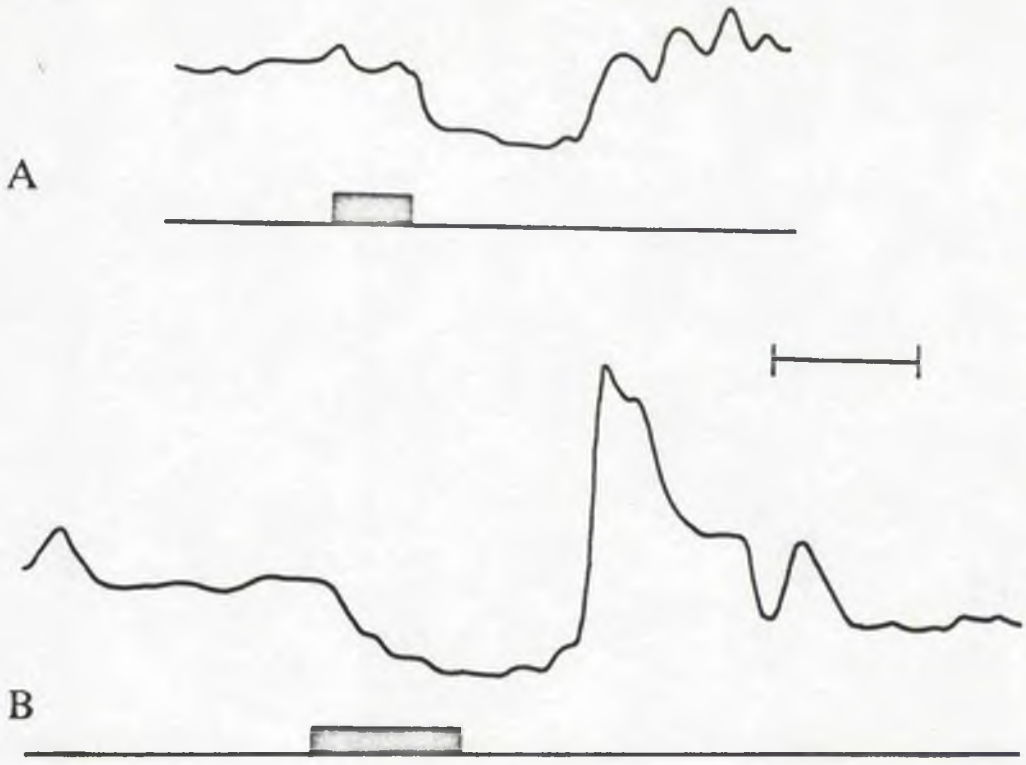
Stimulation of quiescent preparations also elicits an increase in the length of the radials (Fig. 2.9) and the recovery process invariably involves an initiation or increase of spontaneous activity. Once initiated in this way, the spontaneous activity was often found to continue for long periods. Sometimes, a very large recovery contraction was evident, as shown in Fig. 2.9B.

The previous observations all refer to stimulation of the SS1 alone. Fig. 2.10A shows the effect of stimulation of the nerve-net alone at different frequencies. The radials respond with slow contractions. Such slow contractions have

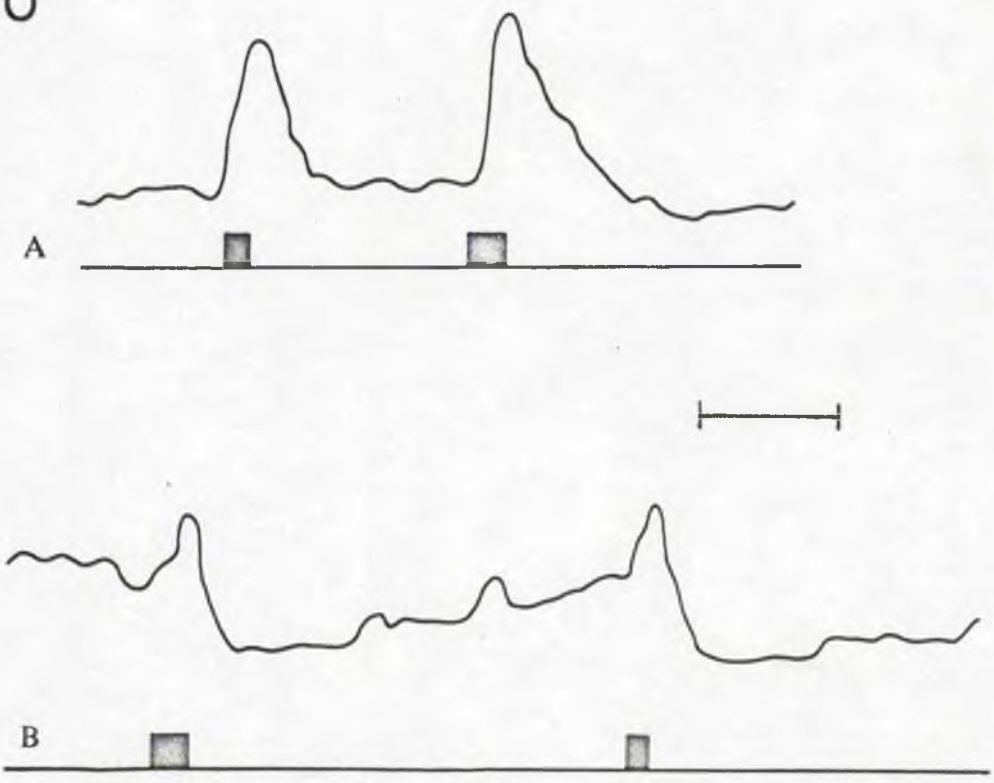
Fig. 2.9. Effect of SS1 stimulation during periods of reduced spontaneous activity. (A) 30 shocks at 1 every 5 sec, (B) 30 shocks at 1 every 10 sec. The recovery process in A involves an initiation of spontaneous activity. Note the very large recovery contraction in B. Time scale = 5 min.

Fig. 2.10. Showing interaction of effects of nerve-net and SS1 stimulation. (A) Electrical stimulation of nerve-net only (stimulating electrode on mesentery); firstly 10 shocks at 1 every 5 sec and secondly 10 shocks at 1 every 10 sec. The radials respond with slow contractions. (B) Stimulation of intact column (nerve-net and SS1 together); firstly 10 shocks at 1 every 10 sec and secondly 10 shocks at 1 every 5 sec. Note reduced slow contraction followed by increase in length and reduction of spontaneous activity. All results from the same preparation. Time scale = 5 min.

2.9



2.10



been described in the oral disc of Metridium senile (Batham & Pantin, 1954) where they occur at stimulus frequencies between 1 shock every 5 sec and 1 every 20 sec. Batham & Pantin pointed out that the slow contractions might arise from action of the endodermal radial fibres of the mesenteries (transverse mesenterics), but they believed that this was unlikely, as these muscles are attached only where the mesenterics insert onto the oral disc, and buckling was not seen to accompany contraction. Another similarity between Tealia and Metridium is that the oral discs of both show small but clear fast contractions to every shock following the first of a series at high frequency (greater than 1 shock every 2 sec). Recordings of electrical activity from the oral disc of Tealia reveal that muscle action potentials are not recorded after the first (facilitating) shock, but may be observed succeeding all subsequent shocks in the high-frequency series.

The results above indicates that low-frequency nerve-net activity induces slow contraction of the radials, whereas excitation of the SSl at identical frequencies brings about relaxation. Presumably, one would expect that simultaneous excitation of these systems would bring about a conflict situation. Fig. 2.10B demonstrates this effect in the same preparation as that used in Fig. 2.10A, Simultaneous activation of the two conduction systems was achieved by placing the stimulating electrode on the intact column and administering shocks atva voltage marginally greater than the threshold for the SSl. The results of two different frequencies of stimulation are shown. Note that in both cases the radials contract during excitation, but these

contractions are much reduced in comparison with those seen in Fig. 2.10A, where identical stimulation parameters were applied to the nerve-net alone.

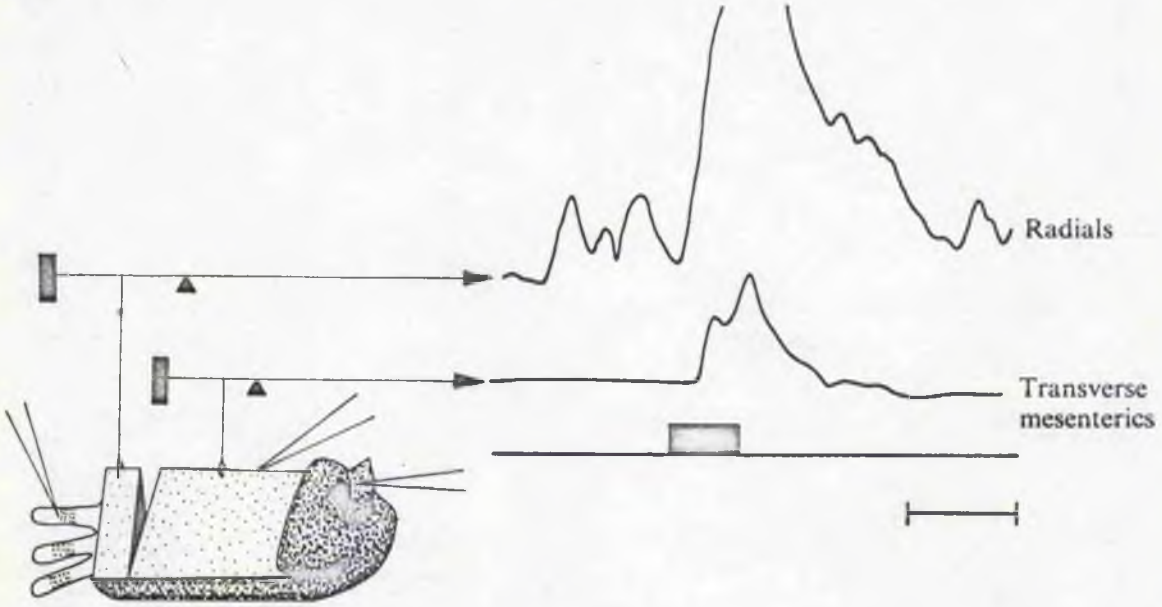
This points to an inhibitory action by the SS1 on those slow contractions induced by nerve-net stimulation. Succeeding the contractions, a lengthening of the radials is also observed, and this is accompanied by a reduction in spontaneous activity, followed by a slow recovery process to the resting length. These results may be interpreted as showing that the excitatory action of the nerve-net precedes the inhibitory action of the SS1. They also indicate that the SS1 can elicit an inhibitory effect on both spontaneous and induced slow contractions.

It was pointed out by Batham and Pantin (1954) that results obtained with radial muscle preparations could be liable to misinterpretation, owing to the presence of endodermal muscles in the oral disc. It proved impossible to remove these muscles without damaging the ectodermal radials. One therefore has to examine their effects on the preparation by indirect means. The endodermal circular muscles of the oral disc are unlikely to modify the kymograph recordings, since these muscles act at 90^{o} to the radials. The transverse mesenterics, however, act in the same direction as the radials, and could clearly modify the recordings of radial activity. To investigate this, a double preparation was employed, as shown in Fig. 2.11. The mesenterics were not excised and an additional isotonic lever was attached by a thread to a mesentery, near its insertion to the oral disc. This permits one to record the activity of both the transverse mesenterics and the oral disc simultaneously.

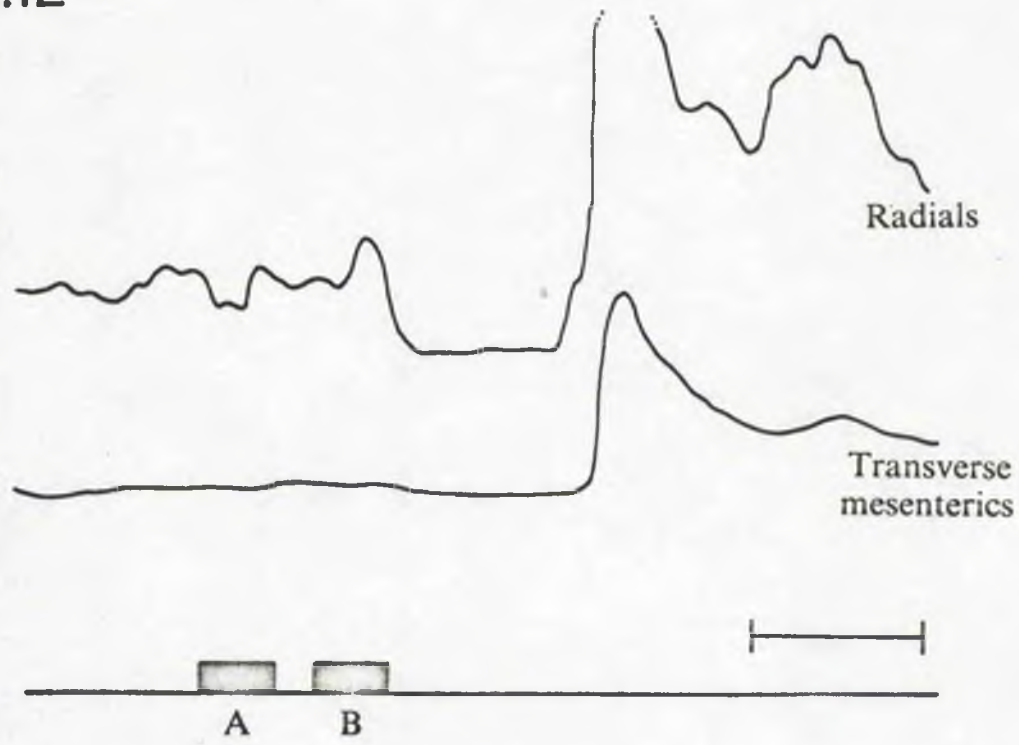
Fig. 2.11. Response of double preparation to nerve-net stimulation. The preparation is shown with a stimulating electrode on a mesentery (to excite the nerve-net) and on a flap (to excite the SS1). Thirty shocks to the mesentery at 1 every 15 sec elicit a slow contraction from both the oral disc radials and the transverse muscles of the mesentery. Time scale = 10 min.

Fig. 2.12. Response of double preparation to SS1 stimulation. Firstly the flap was stimulated below SS1 threshold (A) and no response was elicited. Then stimulation was applied above SS1 threshold (B), causing inhibition of radial activity but showing no clear action on the transverse muscles. Both muscle groups, however, showed a recovery contraction. In both cases 30 shocks at 1 every 15 sec were applied. Time scale = 10 min.

2.11


Radials
Transverse
mesenterics

2.12


Radials
Transverse
mesenterics
A
B

Excitation of the nerve-net alone, by electrical stimulation of either a mesentery or the column under a flap, results in a slow contraction of both muscle groups (Fig. 2.11). The contraction of the radials was large enough to cause the writing point of the lever to overrun the top of the record. In contrast, the contraction of the transverse mesenterics appears relatively weak.

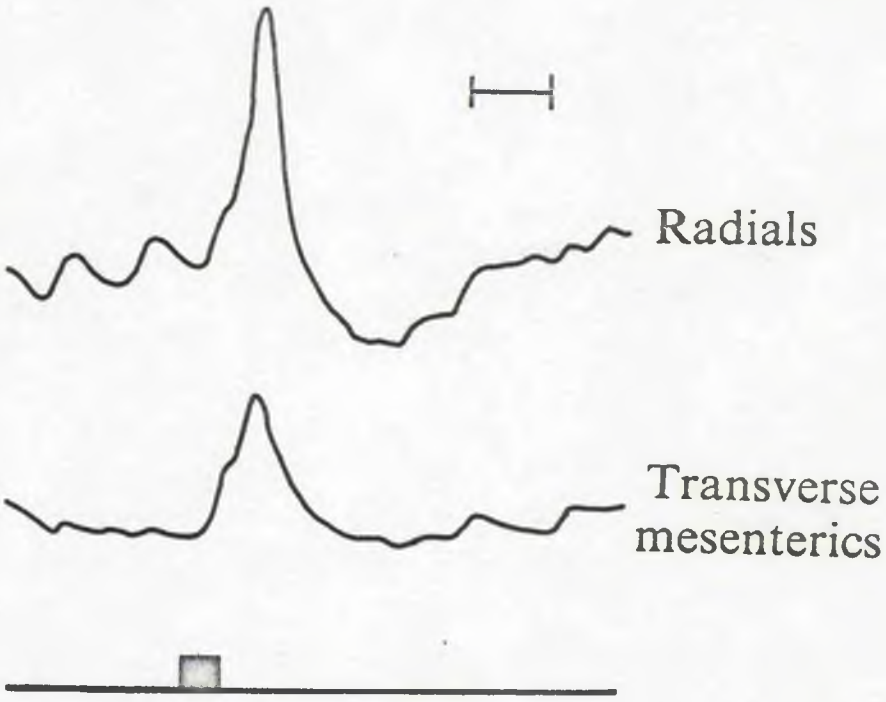
In this type of preparation the effect of stimulating an SS1 flap is depicted in Fig. 2.12. The flap was stimulated firstly just below and secondly just above SS1 threshold. Note the lack of any type of response in the absence of SPIs, indicating that the inhibitory action is not due to stimulation of a conduction system with a threshold lower than that of the SS1. The typical inhibitory effects occur in the radials following the second series of stimulation. In contrast, the transverse mesenterics show no obvious response, implying that the inhibitory effect of the SS1 acts on the radials only. The recovery process shown in Fig. 2.12 surprisingly involves both parts of the preparation, which respond with large contractions. The pathway for this effect is not known, although it is possible that spontaneous excitatory activity in the through-conducting nerve-net may be responsible. Whatever the cause of this recovery contraction, it should be pointed out that normally the transverse mesenterics do not respond to SS1 stimulation in this way.

Stimulation of both nerve-net and SS1 in the double preparation produces the responses shown in Fig. 2.13. The radials demonstrate the effects already noted in Fig. 2.10; i.e. a slow contraction during stimulation, reduction of spontaneous contractions, and lengthening on completion of

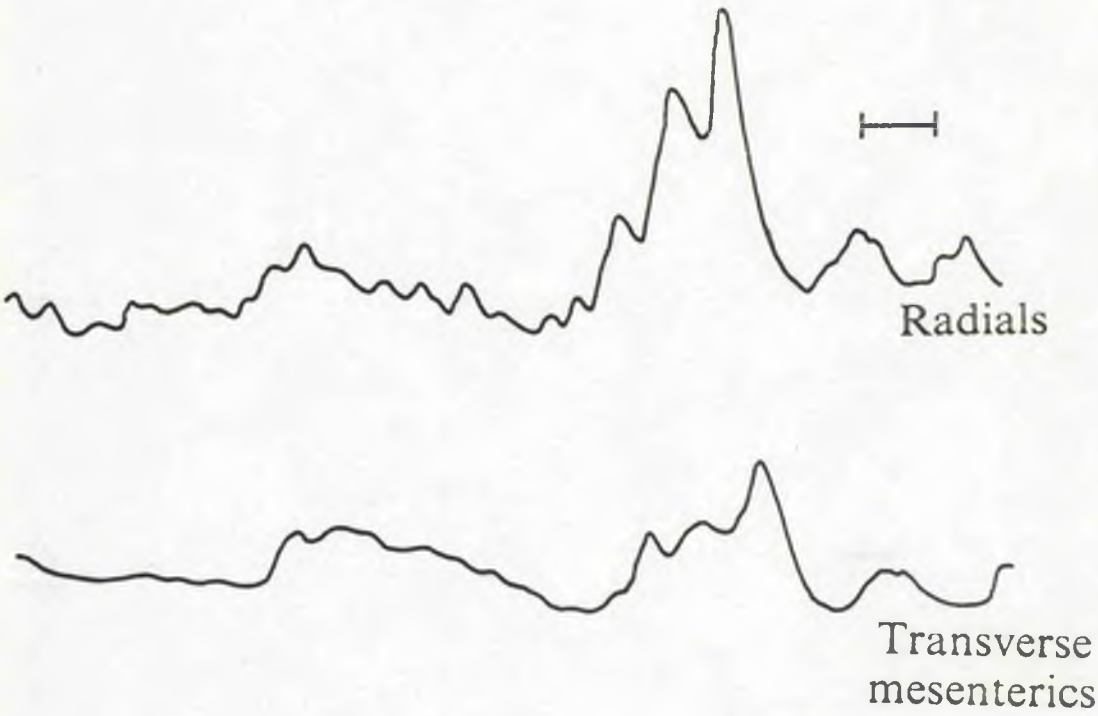
Fig. 2.13. Response of double preparation to simultaneous stimulation of the nerve-net and SS1. The stimulating electrode was attached to the intact column and 5 shocks at 1 every 30 sec were applied. The radials show a similar response to that seen in Fig. 2.10B. The response of the transverse mesenterics, however, does not appear to be modified by this type of stimulation. A slow contraction occurs during stimulation, but there is neither an overall lengthening nor a recovery phase subsequent to stimulation. Time scale = 5 min.

Fig. 2.14. Spontaneous contractions in an unstimulated double preparation. Occasional, irregular spontaneous contractions seem to occur simultaneously on both the radials and the transverse mesenterics. In the radials, however, one can also observe small periodic contractions that are not encountered in the transverse mesenterics. Time scale = 5 min.

2.13


Radials
Transverse
mesenterics

2.14


Radials
Transverse
mesenterics

the initial contraction. This slow contraction is considerably reduced compared with that evoked by nerve-net stimulation alone (see Fig. 2.11). Note that in the transverse mesenterics there is no modification of the slow contraction, as it appears to have a similar amplitude to that seen in Fig. 2.11. There is also no recovery process to accompany that encountered in the radials.

The inhibitory response of the radials shown here is of additional interest, as it was induced by only 5 shocks at a frequency of 1 every 30 sec. This may be interpreted as demonstrating that only a few SPIs at very low frequencies are required to elicit a similar effect to that produced by many SPIs at higher frequencies. It is obviously difficult to make direct comparisons between different stimulation parameters because of the considerable variations encountered between individual preparations, and the irregular nature of the spontaneous activity. One might expect, however, that in the intact animal relaxation of the oral disc would be brought about by relatively low-frequency firing of SPIs. This is deduced from observations on intact animals and preparations responding to chemical stimulation, and will be discussed in more detail later.

A common feature of the oral disc preparations is the presence of spontaneous activity in the radials. This periodical spontaneous activity seems to be a specific property of the radial muscles as it is not encountered in the transverse mesenterics. Fig. 2.14 demonstrates the spontaneous activity in an unstimulated double preparation. Note that both the radials and the transverse mesenterics show occasional, very irregular spontaneous contractions, but in the radials a

more rhythmic series of smaller contractions, occurring at a higher mean frequency, appears to be superimposed on the underlying irregular contractions. The nature of the occasional irregular contractions is unknown, but they may be associated, in some way, with activity in the through-conducting nerve-net, as they seem to occur simultaneously in both muscle group. The source of this spontaneous nerve-net activity may arise from stretching effects caused by the pulling of the isotonic levers.

Similarly, the smaller periodic contractions encountered in the radials alone may be a response of these muscles to stretching. In this case, the contractions appears to be local and perhaps myogenic, since no electrical activity associated with them can be recorded from the tentacles nearby. This type of spontaneous activity may be an exclusive property of preparations, since it is not possible to visually detect these movements in the oral disc of intact animals.

Action of Food Extract

It is known that dissolved food substances contacting the column of Tealia elicit SS1 activity and expansion of the oral disc in the intact animal (McFarlane, 1970). The results of electrical stimulation of oral disc preparations suggest that addition of food extract to the preparation should induce an inhibition of spontaneous activity and lengthening of the radials.

The extract was prepared from molluscan (Mytilus) tissue by the method described in Part Three. No spontaneous SS1 activity was detected in a resting preparation, but shortly after addition of the extract a series of SPIs was

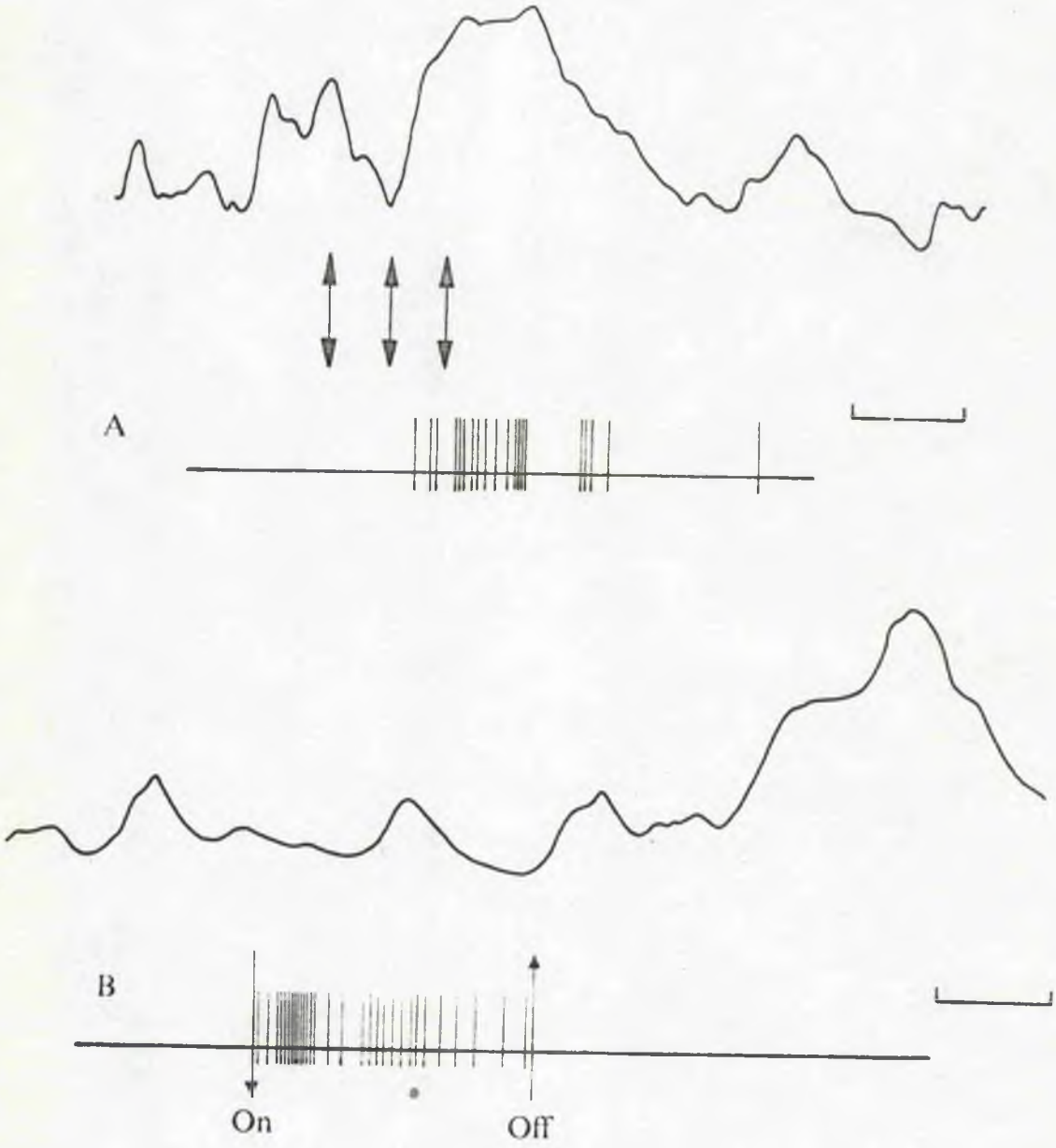
recorded. During these experiments the extract was added directly to the bath and the water supply was turned off temporarily to prevent extract from flowing away from the preparation. The occurrence of each monitored SP1 was marked directly on the kymograph with an event marker. Fig. 2.15A shows the results of such an experiment, in which the monitored SP1 activity is correlated with the muscular activity of the preparation.

The predicted effects of inhibition of spontaneous activity and lengthening of the radials appear to be completely contradicted; instead a large slow contraction follows addition of extract. In one preparation, where a very diluted extract was added to the bath, a short-lived relaxation was observed, but this was soon succeeded by a slow contraction. This paradoxical situation may be explained by the fact that the extract has access to all parts of the preparation. This would presumably elicit a true feeding response involving contraction of the tentacles and oral disc. The pre-feeding response, in which SSl activity occurs accompanied by oral disc expansion, is believed to be elicited by dissolved food substances contacting the column of Tealia (McFarlane, 1970). Therefore, to obtain the predicted effects on the preparation, a method of local application of the extract to the column would have to be devised.

This problem was overcome by using a new technique. A conventional polythene suction electrode was filled with food extract and was then attached to the column in the normal way. The electrode could now be used both as a normal stimulating electrode and as a strictly localized applicator of

Fig. 2.15. The response to dissolved food substances. In each case the upper line shows the activity of the radial muscles and the lower line shows SS1 activity (the length of this line indicates the period of SS1 monitoring and the vertical lines show the position of SPIs). In A *Mytilus* extract was simply placed in the bath (the arrows show three successive applications of equal quantities of extract). No SPIs were seen before application of extract. The radials contracted, possibly due to stimulation of other systems by the extract. In B the extract was applied to a small part of the column only (see text for description of technique). The arrows show the time of application and removal of the stimulus. SPIs were seen only during the period of stimulation. The radials show a slight relaxation followed by a delayed recovery contraction. Time scale = 10 min.

2.15



extract. This type of electrode, capable of both chemical and electrical stimulation, will be referred to as the CSE (chemical-stimulating electrode).

The best results were obtained with electrodes of approximately 1 mm diameter at the tip. A total of about 0.8 ml of extract could be contained in the electrode, presumably ensuring that sufficient amounts of stimulating chemical were available. The extract obtained from the Mytilus tissue was very concentrated, usually being diluted with an equal volume of sea water. This would ensure an active response in the SSl when applied to the column ectoderm. In all experiments the CSE was attached to within a few millimetres of the base of the column.

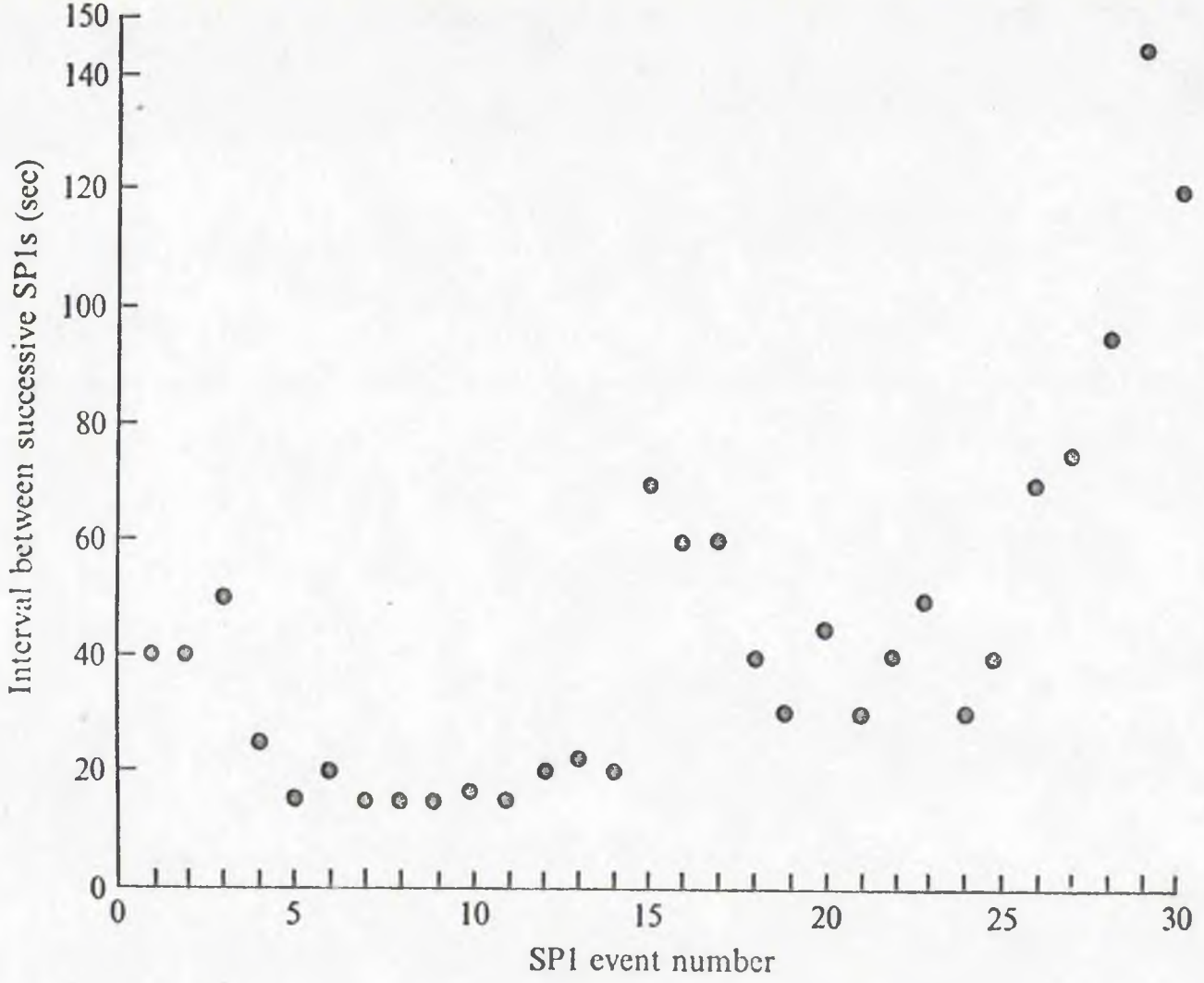
The results obtained are shown in Fig. 2.15B. Once again, no SPls were observed during a 10 min monitoring period prior to electrode attachment. The first SPl was seen 40 sec after attachment of the CSE. This delayed effect may be due to the fact that during application of the CSE, sea water was slowly sucked into the electrode to prevent leakage of the extract into the bath. The 40 sec delay may represent the time taken for the stimulating chemicals to diffuse through the sea water and reach the surface of the column. During stimulation considerable suction was applied to the CSE in order to form a tight seal between the wall of the electrode and the column ectoderm. It is, therefore, unlikely that the extract could escape into the bath during this period. Control experiments, with electrodes containing sea water only, verified that the evoked SPls are not a result of the applied suction.

During the 24 min of stimulation 30 SPls were seen, and on removal of the CSE all activity in the SS1 ceased immediately. The kymograph results are again not as clear as those obtained with electrical stimulation, but the contraction effects observed in Fig. 2.15A are absent. This is good evidence that such effects are due to the action of food extract on the oral disc and tentacles. In Fig. 2.15B one can observe a slight inhibition in the spontaneous activity of the oral disc, accompanied by a slight lengthening. The only contraction that occurs during SS1 activity arises during a period when firing frequency fell below 1 SP1 per min. This may have permitted the spontaneous excitation to overcome inhibition. The large contraction that followed removal of the CSE may represent the recovery process described in previous preparations. It is possible, however, that it may be due to a leakage of food extract into the bath during removal of the CSE, although every care was taken to ensure that this did not happen.

Inhibition may be less clear in this type of experiment because of the very low mean frequency of firing of SPls. The experiment depicted in Fig. 2.15B reveals an average SP1 frequency of only 1 every 45 sec, although the maximum frequency in the early stages of the response was 1 every 15sec. It was noted in earlier experiments that very low-frequency electric l stimulation of the SS1 has only a slight effect on the activity of the radials. Thus, at stimulation frequencies of 1 SP1 every 60 sec the inhibitory response becomes increasingly difficult to observe (see Fig. 2.7B). At frequencies of 1 SP1 every 30 sec they are more obvious (see Fig. 2.5B) although the effective

Fig. 2.16. Showing possible sensory adaptation in the response to dissolved food substances. These results are taken from Fig. 2.15B and show the gradual increase in pulse interval of the 30 SPIs elicited during the 24 min of stimulation. Application of extract was restricted to a small area of the column, and although stimulation was continuous the frequency of evoked SPIs fell considerably during the response.

2.16


Interval between successive SP1s (sec)
150
140
120
100
80
60
40
20
0
0
5
10
15
20
25
30
SP1 event number

frequency in the intact animal may be lower.

The sensory response in preparations seems little different from that described in intact animals (McFarlane, 1970). The intervals between successive SPIs for this response are shown in Fig. 2.16. This graph is of considerable interest as it contains the first information relating to activity frequency in a chemosensory response of a sea anemone. One important feature of this graph is that the response appears to show sensory adaptation in the presence of excess stimulating chemicals, although this sensory adaptation is on a much extended time-scale compared with sensory responses of higher animals. In Part Three, experiments employing two types of stimulating electrode will reveal whether this adaptation is local to the point of stimulation or involves fatigue in the SSl.

DISCUSSION

These studies on the activity of the oral disc in Tealia felina have provided some insight into the mechanisms of control and coordination in the muscles involved. The functioning of the radial muscles, and the possible underlying mechanisms responsible for their activity, will be discussed in this section.

Innervation of the Ectodermal Radials

The radial muscles of the oral disc, together with the tentacle longitudinals, are the only ectodermal muscles to be found in Tealia. This applies to the majority of sea

anemones (Stephenson, 1928) though a few, such as Gonactinia prolifera, possess muscles and an associated nerve-net in the ectoderm of the column (Robson, 1971).

The presence of a nerve-net in the ectoderm of the oral disc and tentacles has been clearly established in many species of sea anemones by several authorities (Hertwigs, 1879-80; Robson, 1963; Batham, 1965; for further references see Stephenson, 1928). It is, however, debatable whether such a nerve-net exists in the ectoderm of the column and pedal disc. The Hertwigs (1879-80) in their extensive histological studies on actinians, stated that a nerve-net existed in these regions, although it was much sparser than that found in the oral disc and tentacles. More recent work by Leghissa (1965) suggests the presence of a superficial nerve plexus lying throughout the ectoderm in Actinia equina. He states that the ectodermal nervous system of the column and pedal disc is fundamentally similar to that found in the tentacles and oral disc, but sparser. Most modern authorities, however, have failed to detect nerve elements in the ectoderm of the column and pedal disc. Batham (1965) in a histological study of Mimetridium cryptum concluded that it was impossible to positively identify any nerve cells in the column ectoderm of this species. It is obvious from these structural studies that there is no unequivocal evidence, to date, to enable one to establish whether the SSl is a nervous or non-nervous system.

The ectodermal nerve-net of the oral disc and tentacles may be connected to the endodermal through-conduction system at the junction of the oral disc with the mesenteries. In Mimetridium, Batham (1965) established that

individual neurites may pass from the endoderm of the mesenteries to the ectoderm of the oral disc, and through to the tentacles. She suggests that this would provide a direct through-conduction system linking the muscles involved in the protective withdrawal response.

Responses of the Radials

This study has revealed that the radials show several responses, and these will now be discussed separately.

i) Fast Contractions: The protective withdrawal response in Tealia can be elicited by strong mechanical stimulation. Behavioural observations indicate that this involves a retraction of the oral disc due to contraction of the endodermal retractors. The ectodermal muscles of the oral disc and tentacles also seem to be involved in this response. The radials contract rapidly to cause a decrease in diameter of the oral disc, and an associated contraction of the tentacle longitudinals causes shortening of the tentacles. Once the delicate tentacular crown has been withdrawn the endodermal sphincter contracts, in a perfectly coordinated movement, to constrict the upper part of the column and effectively shield the oral disc from the exterior.

These symmetrical fast contractions can also be obtained in the isolated radial muscle preparations. The fast contractions in the radials of Tealia are similar to those encountered in Metridium, as described by Batham & Pantin (1954). The nature of the fast contraction has been described by Pantin (1935a) in his classical studies on the quick response in Calliactis parasitica. Typically, a single pulse in the nerve-net supplying these muscles does not directly elicit

a contraction in the muscle concerned but, somehow, facilitates the response in such a way that subsequent pulses, arriving before the decay of facilitation, will elicit fast contractions.

Electrical activity associated with this response was first recorded in the tropical sea anemone Calliactis polypus by Josephson (1966). He recorded large pulses (probably muscle action potentials) preceding fast contraction in the marginal sphincter and tentacles of C. polypus. In Metridium too, muscle action potentials are recorded preceding a similar response in the mesenteric retractor, but in addition small pulses are detected which could represent activity in the through-conducting nerve-net innervating the retractors (Robson & Josephson, 1969). Similar small pulses have been recorded from the mesenteries of the burrowing sea anemone Calamactis praelongus (Pickens, 1969). and from the tentacles and sphincter of Calliactis parasitica (McFarlane, 1969b). Usually, a small pulse is seen to follow each of a pair of electrical stimuli, but the muscle action potential and subsequent fast contraction occur only after the second (facilitated) pulse.

ii) Slow Contractions: In contrast with fast contractions, no muscle action potentials have been recorded in association with slow contractions. These slow responses seem to account for most of the neuromuscular activity in sea anemones. Needler & Ross (1958), in their studies on the neuromuscular activity of C. parasitica came to the conclusion that slow contractions constitute the basic response of all actinian muscles, and that fast contractions are superimposed upon, and perhaps developed from, the more primitive and general slow contractile mechanisms. Thus, the quick facilitated contractions, observed in muscles innervated by the through-conduction system, would appear to be specialized activity associated with the protective withdrawal response.

The slow contractions in the radials of Tealia are elicited by stimulation at frequencies below those which produce fast contractions. There is no evidence to suggest that fast and slow contractions are mediated by different nerve-nets, since both responses are obtained at the same threshold of stimulation (although this, in itself, does not conclusively rule out the possibility of dual innervation). Furthermore, there is no histological evidence that two muscle types might be responsible for the presence of two different responses in the same muscle field. Batham & Pantin (1954) were of the opinion that fast and slow contractions could only be explained by assuming that the same muscle fibres can contract in both fashions.

Obviously, the evidence to date on the mechanisms controlling symmetrical fast and slow contractions does not allow one to determine whether this double action is a property of the muscle fibres themselves, or is due to different muscle fibres, or dual innervation, or even a combination of these factors.

iii) Local Activity: In addition to these symmetrical responses the radials, and indeed most other muscle groups, seem capable of localized asymmetrical contractions. These local responses are particularly important in feeding activity, especially in anemones like Tealia that lack cilia for transference of food from tentacles to mouth.

My own observations on feeding activity in Tealia indicate that local activity of the oral disc and tentacles is mainly responsible for food conveyance, although symmetrical contractions of the oral disc often accompany the final engulfment of large pieces of food. The feeding response seems

to involve contact of tentacles or oral disc with the solid food, followed by discharge of cnidae and adherence of the food to the tentacles. The tentacle (or tentacles) initially involved in the capture of the food starts to bend towards the mouth. This, presumably, involves a unilateral contraction of the tentacle longitudinals on the side nearest the mouth. As the food is conveyed across the oral disc in this manner, other tentacles in close proximity to the food are often recruited to assist its passage towards the mouth. This is often accompanied by a local contraction of the oral disc radials which tends to bring the lip of the mouth and the food-bearing tentacles closer together. This localized response of the oral disc musculature has also been noted in Stoichactis (Jennings, 1905), Cribrina (Gee, 1913) and in certain corals (Carpenter, 1910). Parker (1917) mentions that localized oral disc contractions also occur in Metridium, but they constitute a relatively insignificant response in the feeding behaviour of this anemone as ciliary action is normally employed.

The feeding response in actinians is usually elicited by a combination of chemical and mechanical stimuli, although in starved anemones the threshold of the response may be so low that mechanical stimulation alone is sufficient to initiate it (Pantin & Pantin, 1943). This feeding activity may involve the ectodermal muscles alone; excitation of the tentacle longitudinals appearing as local tentacle shortening and bending, and activity in the oral disc radials appearing as local contractions and a raising of the oral disc margin. The contractions seen in Fig. 2.15A, in which food juice was allowed to come into direct contact with the tentacles and oral disc, probably represent such local activity, In

this case, although solid food did not make contact with the oral disc, the chemical stimulation provided by the food juice may have lowered the threshold to mechanical stimulation to such a degree that the mere stretching effect of the isotonic lever would have been sufficient to induce a contraction in the radials.

Local contractions are important since they enable one part of the tentacular crown to be involved in capture and manipulation of food whilst allowing the other part to remain expanded, ready for further food capture. These local responses were explained by Pantin (1935a) on the basis of interneural facilitation. Pantin implied that lateral spread in the oral disc was dependent upon facilitation between neural elements. These deductions, however, were made from observations employing muscular contraction as the measured output of the system. A different picture of the nature of local contractions has emerged since the advent of electrical recordings from sea anemones by Josephson (1966).

The chief criticism of Pantin's ideas concerning local activity is directed towards his suggestion that both local and through-conducted responses were due to activity in a single nerve-net. The more recent evidence against this idea includes the following:

a) A single shock of the lowest effective intensity always gives twitching of widely spread tentacles in Calliactis polypus (Josephson, 1966). A close second shock gives overall fast contraction. These are responses that might be associated with a through-conduction system.

b) Strong shocks and mild mechanical stimulation give local contractions which are, to some extent, graded with

intensity of stimulation (Josephson, 1966). These local responses are not associated with through-conduction as they occur at a higher threshold. This suggests that local responses of the oral disc involve a system separate from the through-conduction system.

c) A similar situation is apparent in Cerianthus membranaceus (Horridge, 1958). In this case, however, it was found that the local system had a lower threshold than the through-conduction system.

d) A single shock to isolated tentacles from Radianthus sp.?, Tealia felina, and Anemonia sulcata produces a contraction which is graded with the intensity of stimulation (Davenport, 1962). This observation indicates that such activity must be separate from the protective withdrawal response mediated by the through-conduction system.

e) Recordings of electrical activity in the oral disc of Tealia felina (personal observations and McFarlane, 1969a) show that a single shock (just above nerve-net threshold) to a tentacle on one side of the oral disc produces a single pulse, associated with the nerve-net, that may be recorded from a tentacle on the opposite side of the oral disc. This is not a case in which the entire system has been facilitated, however, since the only tentacles to contract are those nearest the point of stimulation. This implies that both through-conducted and local systems must exist in the oral disc.

f) Muscle action potentials associated with through-conducted activity consist of a single biphasic pulse lasting 50-100 msec. Such a potential is invariably accompanied by a symmetrical fast contraction, which may be very weak and give little visible shortening of the tentacles. In contrast, local

contractions are accompanied by a series of small pulses with a very different appearance from the biphasic muscle action potentials. The duration and amplitude of these local pulses depends on a number of parameters, such as stimulation intensity, distance from the region stimulated, and past history of stimulation. This type of complex activity is shown in Parts One and Three.

g) Recordings of electrical activity in Anemonia sulcata (McFarlane, 1969a) indicate that through-conducted activity is present in this animal, although all behavioural responses appear to be local (Pantin, 1935b). Again, this points to the presence of two conduction systems.

h) Magnesium anaesthetization of Anemonia gives results suggesting that the local system is non-nervous (McFarlane, 1969a). Fast contraction responses disappear after 10 min, SPIs disappear after 30 min, but local responses persist for upto 45 min from the beginning of anaesthetization. This suggests that the local system may reside in the muscles themselves, indicating the involvement of either mechanical or myoid conduction.

All the evidence presented above suggests that local disc contractions involve a system separate from the through-conduction system. Until direct evidence can be obtained it may be wise to assume, on the principle of economy of hypothesis, that local activity is the result of mechanical or myoid conduction between fibres in the ectodermal muscle sheet. Presumably, restrictions on spread of conduction are somehow imposed by the radial orientation of the fibres. Should observations on the fine structure of the oral disc reveal the presence of tight junctions between muscle cells,

then this might indicate that conduction is electrical (myoid conduction). Until unequivocal evidence for such tight junctions can be obtained, however, it is probably simpler to assume that local conduction between muscle fibres is mechanical.

iv) Spontaneous Activity: The radial muscle preparations also show spontaneous activity. As mentioned earlier, there appear to be two types of spontaneous activity. One occurs only occasionally and produces very irregular, slow contractions. These contractions are seen both in the radials and the transverse mesenterics of unstimulated preparations (see Fig. 2.14) and could possibly be due to activity in the through-conduction system. Certainly, similar types of slow contraction can be elicited in both muscle groups by stimulation of the nerve-net at low frequencies (see Fig. 2.11). In resting preparations these occasional contractions may arise from stretching effects due to attachment of the isotonic levers. In Galliactis parasitica mechanical stimulation seems to elicit a burst of nerve-net pulses that results in contraction (Passano & Pantin, 1955).

Batham & Pantin (1954) came to the conclusion that spontaneous impulses undoubtedly passed over the through-conduction system in unstimulated specimens of Metridium senile. The natural frequency of these spontaneous impulses was estimated indirectly by applying single shocks at low frequency to the through-conduction system and noting the percentage of such shocks that elicited fast retractor responses. Since the duration of the facilitatory effect for this response was known, it was possible to estimate the frequency of spontaneous activity in the through-conduction

system. This estimate was, of course, based on the assumption that a single applied pulse would act in conjunction with a spontaneous pulse in the nerve-net to elicit a facilitated fast contraction in the retractors. Such an estimate does not take into account the possible occurrence of "after-discharges" as a result of single shocks. As Pantin (1935c) points out, however, supernumerary contractions may result from a number of causes and seem to occur as a result of two or more stimuli, never single shocks.

Recordings of electrical activity in Calliactis parasitica seem to confirm Pantin's observations on the phenomenon of supernumerary contractions (McFarlane, 1973a). Such contractions were only obtained in certain animals, and were recorded as additional muscle action potentials in response to paired stimuli. Occasionally, these "after-discharges" may be elicited by single stimuli (McFarlane, personal communication), but this is a very rare phenomenon, possibly resulting from an interaction between electrical and mechanical stimuli. This suggests that such responses are unlikely to invalidate Batham and Pantin's results.

A more important objection to these indirect estimates, however, is suggested from Ross' (1952) observations on fast responses to single stimuli in Metridium senile. He ventured the possibility that pulses arising from spontaneous activity in the nerve-net had different properties from those induced by electrical stimulation. This hypothesis was deduced from observations on untreated animals in which it was noted that when fast contractions did occur to single shocks practically all these contractions occurred "on the stimulus", whereas only 3% showed a contraction delay of upto

3 sec (i.e. representing the decay time of the facilitatory effect evoked by the applied shock). Ross, therefore, suggested that since spontaneous and delayed contractions were so infrequent, then perhaps spontaneous impulses in the nerve-net were only capable of facilitating the quick response, rather than directly evoking it. This would imply that there were separate channels of excitation responsible for the facilitating and the excitant phases of the response. The possibility of distinct facilitatory and excitatory modes of stimulation in the nerve-net should obviously be considered when discussing spontaneous activity.

McFarlane (1973a) has pointed out that it is difficult to test whether spontaneous impulses are only facilitatory as it is impossible to accurately predict the occurrence of these pulses. It would, therefore, prove difficult to stimulate the nerve-net immediately before the arrival of a spontaneous impulse in order to observe whether a fast contraction occurs or not. It was noted, however, that delayed contractions seemed relatively common in C. parasitica suggesting that spontaneous impulses were capable of evoking the contraction directly. Even so, the electrical activity recorded during one of these delayed contractions revealed that although the single shock applied to the column was followed by a single pulse in the nerve-net, there was no equivalent pulse directly preceding the delayed muscle action potential. Such a response might be evoked by a local spontaneous impulse not detectable by the recording electrode, or alternatively some sort of delaying effect might have occurred in the muscle itself.

Obviously, the mechanism by which contractions occur to single shocks is still poorly understood, and one should be

wary in the interpretation of results employing indirect methods to find the frequency of spontaneous activity in the nerve-net. From such methods Batham & Pantin estimated spontaneous frequencies ranging from 1 pulse every 2 min to 1 every 12 min and deduced from Ross' results a range from 1 every 7 min to 1 every 70 min. From direct observations of spontaneous electrical activity, McFarlane (1973a) estimated a slightly higher average frequency for C. parasitica of 1 pulse every 3 min, although he has shown that the spontaneous nerve-net pulses occur in bursts of much higher frequency. Consequently, these direct studies generally agree with the indirect estimates of frequency deduced by Batham & Pantin for Metridium, and confirm the contention that spontaneous nerve-net activity might play an important role in sea anemone behaviour.

Direct evidence that such activity actually causes slow contractions has been shown in Calliactis parasitica (see Part One, and McFarlane, 1973 a,b). These observations indicate that spontaneous high-frequency firing in the nerve-net (approximately 1 pulse every 5 sec) normally elicits a slow contraction of the sphincter. In this case, the number and frequency of impulses is in agreement with the ranges known to elicit slow sphincter contraction (Ross, 1957). Furthermore, spontaneous bursts of nerve-net activity seem to produce large slow contractions in the parietal muscles of Calliactis (McFarlane, 1973b). This directly confirms Batham & Pantin's (1954) proposition that similar contractions in Metridium are caused by these bursts. This nerve-net activity presumably interacts with the naturally-occurring, local spontaneous activity of the separate parietal sectors in

order to initiate a coordinated parietal contraction. This would then inhibit contraction of the circulars, owing to the existence of reciprocal inhibition between these two muscle groups (Batham & Pantin, 1954). Once the coordinated parietal contraction had ceased, a slow contraction of the marginal sphincter would ensue, which in turn would initiate a slow peristaltic wave to pass down the column. In this way, one may appreciate the important role that spontaneous activity in the nerve-net might play in muscular coordination.

These observations all serve to strengthen the idea that spontaneous bursts of activity in the through-conduction system are responsible for the occasional slow contractions observed in Fig. 2.14. The radials, however, show another type of spontaneous activity (not seen in the transverse mesenterics) consisting of small periodic contractions producing cyclical variations about the incidental length of the muscle.

No electrical activity can be recorded in association with these contractions and this would imply that the through-conduction system is not involved. These rhythmic cycles of contraction and relaxation may represent a response of the radials to stretching, and therefore this activity could originate from the muscle fibres themselves. Although it is not possible to visually detect such contractions in the oral disc of intact animals, this does not necessarily imply that they exist only in the preparations. It is conceivable that these contractions are so small that they could not be seen without the necessary magnification afforded by the kymograph system.

They may originate from myogenic activity in the

radial muscle fibres, or from pacemaker activity in a local nerve-net. Although the nature of pacemaker systems has been well studied in scyphomedusae and hydrozoans, relatively little is known about such activity in anthozoans. Pacemaker systems have been studied in the swimming sea anemones, Stomphia coccinea (Robson, 1961c, 1963), and Gonactinia prolifera (Robson, 1971).

In S. coccinea sectioning experiments indicate that some kind of pacemaker system, residing in the middle of the column, is responsible for the swimming activity. Swimming basically consists of a series of abrupt bending movements brought about by an alternation of parieto-basilar contractions at opposite radii (Robson, 1961c). Local parieto-basilar contractions may be produced by single electrical shocks applied to the base of the column of Stomphia (Hoyle, 1960). It is, therefore, possible that each contraction of the parieto-basilars during swimming is due to a single impulse in the pacemaker system. Hoyle believed that because of the great variation in the magnitude of the response to any constant-strength stimulus, it was unlikely that the contractions were due to a direct excitation of the muscle fibres. There was also no evidence of direct excitation of the circular muscles that pass between the electrodes and the parieto-basilars. Hoyle concluded that the parieto-basilars were being excited indirectly, via a local nerve-net. Robson (1961c) noted that the pacemaker system seemed to be excited only by shocks delivered in its vicinity, which would suggest that it could not be directly excited by activity in the through-conduction system.

From these behavioural and physiological observations

one might expect to find a specialized region of the nerve-net, associated with pacemaker activity, in the median zone of the column. Histological studies of the nerve-net in Stomphia (Robson, 1963) did not reveal such a region specifically in the median zone of the column. The column as a whole, however, was found to be characterised by the presence of large, multipolar nerve cells not seen elsewhere in the anemone. Such cells have not been encountered so far in the column nerve-net of non-swimming anemones. Sparser and much smaller multipolar cells have been seen in the column of Calliactis parasitica (Robson, 1961a, 1965) but these are probably not comparable to the larger cells encountered in Stomphia. Robson has tentatively suggested that in the absence of physiological information on the function of these large multipolar cells, they may be regarded as constituting the pacemaker system. Obviously, one cannot decide if such cells are responsible for pacemaker activity until direct recordings of electrical activity within these cells have been obtained.

In the majority of sea anemones, however, any rhythmic and spontaneous activity is usually far too slow to have originated in the kind of pacemaker system envisaged for Stomphia. It has been noted by Ewer (1960) that the regular rhythmic contractions encountered in circular muscle preparations of Calliactis parasitica recall the beat of a medusa bell, although on a much slower time scale. His experiments indicate that on completion of a spontaneous contraction an inhibitory effect occurs whose slow decay is reflected in the gradual development of excitability in the circulars. He concludes that such a mechanism could

produce a rhythmical series of contractions if one assumes that a naturally-occurring spontaneous contraction arises from excitation in the nerve-net, but that, in its occurrence, it produces a prolonged inhibition. This would then prevent the development of a further contraction until the inhibitory effect had died away. Ewer also presents evidence to support the idea that the inhibitory effect may occur at the myo-neural junction, but emphasises that his results cannot exclude the possibility that the spontaneous activity of the circulars is myogenic in origin; in which case, electrical excitation would be stimulating extrinsic excitatory and inhibitory pathways. This would parallel the situation encountered in the myogenic heart of vertebrates, where the nerve supply can modify the rhythm of the heart.

All of the above ideas concerning rhythmic spontaneous contractions in sea anemones are based on indirect observations of pacemaker activity. Electrical activity associated with pacemaker systems has been recorded from hydrozoans and scyphozoans, but the only accounts of spontaneous electrical activity in anthozoans are those described in the pennatulid Veretillum cynomorium (Buisson, Tricoche & Franc, 1967) and in the actinian Calliactis parasitica (McFarlane, 1973a).

McFarlane describes spontaneous activity occurring in all three conduction systems (the nerve-net, SS1 and SS2). The SS1 seems to show irregular firing which possibly originates from undetected external stimuli. The SS2 shows rhythmic changes in frequency, and during periods of low-frequency SS2 activity bursts of high-frequency firing occur in the through-conducting nerve-net. It was emphasised, however, that such spontaneous electrical activity in no way implies that genuine

pacemakers are involved, only that this activity does not originate from any obvious external stimulation.

In Tealia felina no electrical activity could be recorded in association with the rhythmic spontaneous contractions of the radials. It is simplest to assume, therefore, that the rhythm is intrinsic, and that this myogenic activity in the radial muscle fibres is modified, in some way, by excitation of the SSl.

It is clear that the origin of spontaneous activity in actinians remains something of an enigma. In my opinion, this is a problem that will not be completely resolved until intracellular recordings from identifiable cells are obtained - a rather daunting prospect at the present time, but one which may be fulfilled with the development of more sophisticated techniques in the near future. In relation to this, it is of interest to note that spontaneous pulses have been recorded from the siphonophore Nanomia bijuga using intracellular techniques (Spencer, 1971). Unfortunately, both procion yellow and Niagara sky blue 6B, when injected iontophoretically were found to diffuse through a number of cells and hence failed to show the exact location of the electrode tip. It is, therefore, impossible to prove that these recordings are intracellular, for it is conceivable that they could be transepithelial pulses. Such pulses have been recorded between the enteron and surrounding water in Hydra (Josephson & Macklin, 1967), although these pulses have the opposite polarity to those recorded in Nanomia.

The difficulty of identification, and thesmall dimensions of the cells concerned are the biggest factors acting against the physiologist who wishes to record from

single cells in these animals. Batham (1965) reports the presence of many stout nerve fibres (often 5-8μm and occasionally as much as 13μm in diameter) situated over the retractor muscles of Mimetridium cryptum. It may, therefore, eventually prove possible to achieve intracellular recordings from such cells in the mesenteries of this, and similar, species. Until this proves possible, one's ideas concerning the origin of spontaneous activity in actinians must be based on indirect observations.

v) Induced Relaxation: The final response observed during this study of the radial muscles is that of induced relaxation. The results show that activity in the SSl is accompanied by a reduction of spontaneous activity and lengthening of the radials. This response is found to occur over a wide range of frequencies (from 1 impulse every 2 sec to 1 every 60 sec) and often shows a slow onset which seems to be dependent on frequency and number of stimuli. The induced relaxation appears to have a relatively long-lasting effect, even after SSl activity has ceased, and the recovery process is also long-lasting, involving a gradual increase in spontaneous activity and shortening of the radials.

On the basis of the evidence outlined in the introduction to this thesis it is probably fair to assume that the SSl is an epithelial conduction system located in the ectoderm. It is interesting to speculate on the mechanisms by which electrical activity in the epithelial cells of the oral disc (SSl activity) can, to some extent, inhibit the activity in the muscle cells beneath them.

To date, the clearest case of electrical inhibition is that of the Mauthner cells in the goldfish (Furukawa &

Furshpan, 1963). Impulses in the thin, helical presynaptic fibres (which form a synaptic link with the axon hillock region of these large Mauthner neurones) cause a hyperpolarizing potential to develop in the hillock region, thus raising the threshold for impulse initiation in the Mauthner cell. As there are also chemically transmitting inhibitory endings nearby, on the cell body of the Mauthner neurone, one can assume that the purpose of the electrical synapse is to produce a rapid inhibitory response.

Recent work by Faber & Korn (1973) has shown that the impulses in the Mauthner cell itself cause electrically mediated inhibition in adjacent neurones. In both cases, the hyperpolarization of the postsynaptic neurone is believed to result from an inward transmembrane flow of the extracellular current generated by the impulse in the presynaptic neurone. This type of response depends on the existence of either high extracellular current densities, or low membrane resistances in the regions involved. Thus, one would expect to find low-resistance pathways in electrical synapses, and this agrees with the findings that their membranes may be fused, or the clefts may be narrower than 20 nm. In contrast, the majority of chemical synapses examined so far appear to be wider than 20 nm.

If ultrastructural studies on the oral disc reveal such a close juxtaposition between the ectodermal epithelial cells and the radial muscle fibres, then electrical inhibition of activity in the radials might be a possibility. One could envisage that extracellular currents, generated by activity in the SSl, would cause a hyperpolarizing potential to develop in the muscle membrane, thus raising the threshold

for initiation of the muscle action potential. Unfortunately, because of the considerable difficulties encountered in ultrastructural studies on coelenterate tissue, it is doubtful if really reliable evidence can be obtained to convince one that such narrow junctions do exist between the two cell types. In addition, one can obviously not tell if electrical inhibition is occurring until intracellular recordings are achieved from the cells concerned.

The distinct possibility that electrical inhibition may not be involved in this case is reflected in the observations that the onset of the inhibitory response is often quite slow (see Fig. 2.5C), and the inhibitory effect appears to be long lasting, even after SSl activity has ceased. Such delayed effects might suggest some sort of chemically mediated inhibition, although it must be stressed that our knowledge concerning these interactions between membranes and molecules is virtually non-existent in this particular case.

The idea that the inhibitory response in the radials may be chemically, rather than electrically, mediated is probably more credible, judging from the evidence at hand. It must be stressed again, however, that the evidence is purely circumstantial. It has been suggested (Mackie & Mackie, 1967) that neuroid conduction in the siphonophore Hippopodius hippopus may somehow involve calcium in the propagated response. If the SSl is a similar conduction system then it is possible that the propagation of SPls involves removal of calcium ions from the extracellular spaces. In the oral disc region this would imply that SSl activity in the ectodermal epithelial cells would effectively remove extra-

cellular calcium from the vicinity of the radials. This removal of calcium ions might then cause a reduction in the spontaneous activity of the muscle fibres, and eventually bring about relaxation.

In support of this idea it has been shown by Ross (1960a) that in calcium-free sea water the activity and responsiveness of column preparations from Calliactis parasitica soon disappear. He noted that in the absence of calcium ions the preparations cease spontaneous activity and relax steadily. In addition, this inhibitory effect seems to occur gradually, until eventually the preparations become fully relaxed and quite inexcitable. If such an effect is common to other muscle groups in actinians then it is conceivable that the inhibitory effect observed in the radials might well involve removal of calcium ions.

Since the only ectodermal muscles in Tealia are those found in the oral disc and tentacles, one would not expect to see an inhibitory effect in other muscle groups, for the SSl is, presumably, exclusively ectodermal. This, of course, raises the question of whether the ectodermal longitudinals of the tentacles also show an inhibitory response. Although direct recordings of tentacular activity were not attempted during this study, it was noted that relaxation of intact animals involved both expansion of the oral disc and extension of the tentacles. This would presumably serve to increase the chances for food capture by increasing the range of the tentacles.

Davenport (1962), in a study on the responses of tentacles to electrical stimulation, noted that isolated tentacles from Anemonia sulcata and Tealia felina showed

considerable variation in spontaneous activity. Some preparations were relatively quiescent, whereas others gave large, slow arhythmic contractions. Isolated tentacles from Radianthus sp.?, however, differed in their activity by giving slow, but distinctly rhythmic, contractions with a frequency ranging from 1 every 2 min 40 sec to 1 every 10 min. He also noted that spontaneous activity often appeared in quiescent preparations after electrical stimulation, a response also shown in the radials (see Fig. 2.9A). Clearly, both radial and tentacle longitudinal preparations show similar responses, and one should perhaps regard both muscle groups as forming a single muscle field.

In addition, Davenport noted that 12 shocks at a high frequency (7 per sec) induced relaxation and reduced spontaneous activity in isolated tentacles of Radianthus. Presumably, all conduction systems in the tentacle were being activated in these experiments, owing to the stimulation technique being employed. In fact, Davenport noted that initially a slight fast contraction occurred during stimulation, and the relaxation response followed this. This is, perhaps, comparable to the response of the radials during simultaneous stimulation of the nerve-net and SSl (see Fig. 2.10B). The observation that higher stimulation frequencies were effective with Radianthus tentacles may be explained by the fact that Davenport's experiments were carried out at 23-24°C.

It is known that the decay of facilitation in sea anemones increases with temperature (Hall & Pantin, 1937; Ross, 1955). This means that the frequency of stimulation required to produce a given response at room temperature has

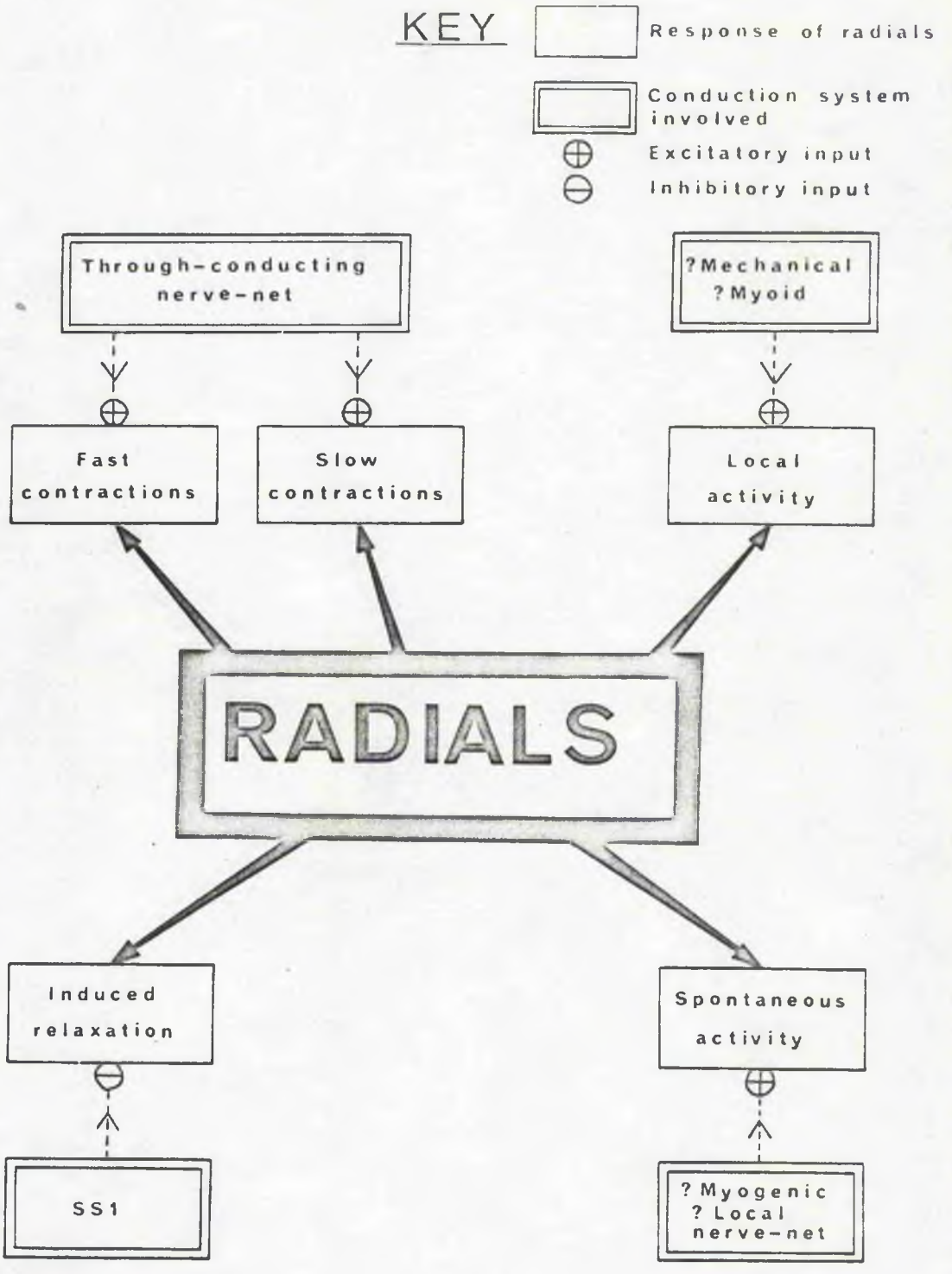
to be increased to produce the same response at a higher temperature. This effect seems to be independent of the normal environmental temperature of the anemone. Hence, muscular facilitation was found to decay at the same rate both in Bunodactis sp? (a tropical sea anemone) and in Metridium senile (a temperate species) when measured at the same temperature (Pantin & Vianna Dias, 1952). Because facilitation decay has a positive temperature coefficient, however, the tropical anemone shows a faster decay of facilitation at environmental temperatures.

This response was also noted in Calliactis polypus (Josephson, 1966; Ross & Sutton, 1963) in which the protective withdrawal reflex could only be elicited at frequencies above 3 shocks per sec at environmental temperatures (26-30°C). In contrast, the same reflex could be elicited in the temperate species Calliactis parasitica at frequencies as low as 1 shock every 2 sec at environmental temperatures (11-15°C). When one considers that physiological acclimation to temperature differences occurs in many different groups of animals, it is, perhaps, surprising to find that tropical and temperate anemones differ so much in the rate of decay of facilitation when each is at its own environmental temperature. This phenomenon may explain the effectiveness of high-frequency stimulation of Radianthus at 23-24°C, for the experiments on Tealia were made at considerably lower temperatures (7-12°C).

These observations seem to confirm that the inhibitory response in both the tentacle longitudinals and the radials has a common basis. Obviously, one can only speculate on the mechanisms involved; but from the evidence at hand I am

Fig. 2.17. Summary of the responses encountered in the radials. These muscles are particularly interesting for they seem to respond to different modes of stimulation in many different ways.

2.17


KEY
Response of radials
Conduction system involved
Excitatory input
Inhibitory input
Through-conducting nerve-net
?Mechanical ?Myoid
Fast contractions
Slow contractions
Local activity
RADIALS
Induced relaxation
Spontaneous activity
SS1
?Myogenic ?Local nerve-net

inclined to favour the idea that induced relaxation may be chemically mediated, perhaps by the removal of calcium ions, as outlined above. One must, however, regard the evidence as insubstantial until better techniques become available to enable one to examine these mechanisms more critically.

A summary of the responses of the ectodermal radial muscles, as revealed by this investigation, is shown in Fig. 2.17. It is remarkable that a single set of muscles can apparently respond in so many different ways to different modes of stimulation. The relevance of this in relation to problems encountered in actinian evolution will be dealt with in the General Discussion.

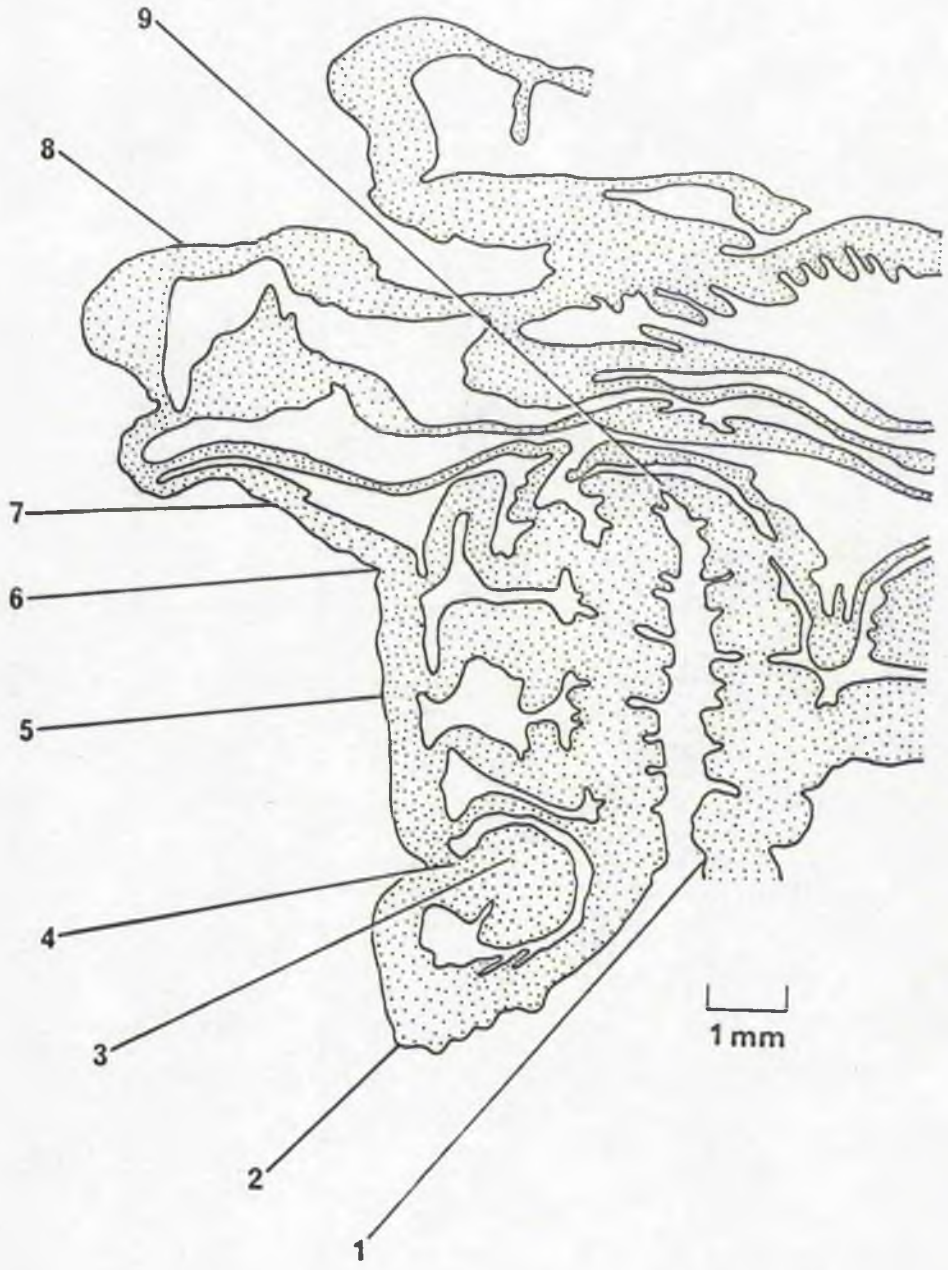
Expansion and Contraction in the Intact Animal.

The results obtained in this study have indicated the way in which the oral disc preparation behaves in response to different modes of stimulation. How can one relate the responses of the preparation to the observed behaviour of the intact animal? What role does the oral disc play in such activities as opening, closing, and expansion?

At first sight it might seem that expansion of the oral disc could not occur by relaxation of the radials alone, and would have to involve additional muscle groups. One might imagine that muscles such as the marginal sphincter and mesenteric retractors would have to relax at the same time as the radials in order to permit expansion of the oral disc. Observations on the anatomy of Tealia, however, suggest that expansion can occur without the involvement of any muscle group other than the radials. Fig. 2.18 is drawn from a light

Fig. 2.18. Vertical section through the marginal region of Tealia. The drawing reveals the anatomy of the marginal region and shows the point of attachment of the sphincter to the body wall. 1, Column wall; 2, collar region; 3, sphincter muscle; 4, point of attachment of sphincter to body wall; 5, upper collar; 6, fosse; 7, capitulum; 8, margin of oral disc; 9, region of thin mesogloea.

2.18



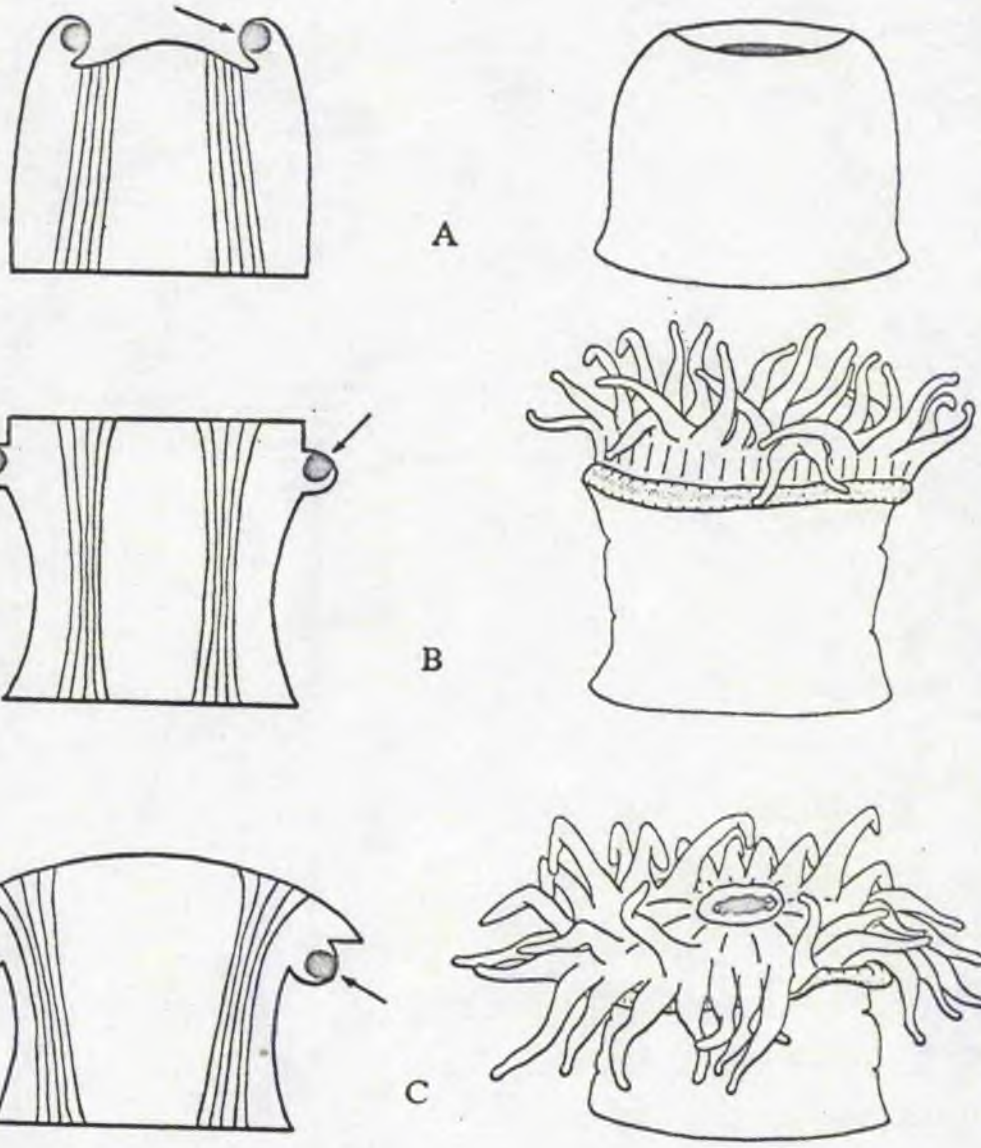
micrograph revealing the anatomy of the marginal region of Tealia. Note that the sphincter lies in a projection of the body wall known as the parapet or collar. This collar structure is encountered in a number of anemones, such as Tealia, Metridium and Actinia (Stephenson, 1928).

The collar encloses on its upper side a groove, known as the fosse, which encircles the marginal region of the anemone. At the lower side of the collar the mesogloea is seen to thin out and form a relatively narrow layer about which the collar can pivot relative to the main part of the column. This point of articulation, denoting a weakened region of the mesogloea, encircles the upper column and is shown diagrammatically in Fig. 2.1 as a trisulcate fold between the collar and column. Between the bases of the tentacles and the collar lies a delicate upper region of the column, the capitulum.

Behavioural observations reveal that as an open anemone relaxes (Fig. 2.19 B to C) there is initially folding at the fosse in such a way that the angle between the capitulum and the collar becomes progressively acute. This tends to make the tentacles point more and more towards the base of the anemone. There is then a folding around the region of thin mesogloea at the base of the collar, thereby causing the collar and sphincter to twist round and downwards. This involves little or no change in the resting length of the sphincter. The diagram shows that the retractors become bent away from the centre of the body, and again little change in length need be postulated. These observations suggest that when an open anemone expands its oral disc no other muscles but the radials need to relax.

Fig. 2.19. Showing relationships of radial muscles, sphincter, and retractors in open and closed Tealia. Diagrams on left show model of relative positions of the muscles and those on right show external appearance. In the model the line at the top represents the radials, the black circle the sphincter and the parallel lines the retractors. The arrow shows the point of attachment of the sphincter to the body wall. (A) closed anemone, (B) open anemone, (C) expanded anemone. Note changes in position of collar region. For expansion from B to C (during the pre-feeding response) there need be no change in the length of the sphincter or retractors, but for opening of a closed anemone (A to B) relaxation of these muscles seems necessary. Relaxation of the radial muscles seems the main cause of expansion (B to C).

2.19



When the anemone closes (Fig. 2.19 B to A) the retractors contract, pull in the oral disc, and alter the angles at the hinges above and below the collar. The arrows in Fig. 2.19 indicate the point of attachment of the sphincter to the collar. This circumscript sphincter is a sharply defined cord of muscle projecting into the body cavity, and is attached only along a narrow line to the body wall (Stephenson, 1928). One can see that when the anemone is in the open positions (Fig. 2.19 B & C) contraction of the sphincter will pull against this point of attachment. Once the collar has rolled over, however, owing to contraction of the radials and retractors (Fig. 2.19A) the sphincter can then act in the direction of the attachment.

This model for expansion and contraction in Tealia is based on the fact that a sea anemone can be regarded as a closed box with an internal hydrostatic pressure slightly positive with respect to the surrounding sea water (Chapman, 1949). One can see from Fig. 2.19 that if a closed anemone needs to open, then both the sphincter and the retractors must relax. This procedure, therefore, involves endodermal musculature, and presumably activation of the SSl alone will be unable to cause relaxation in this case. In fact, it has been noted that closed anemones often fail to open during SSl stimulation (McFarlane, 1970), whereas open anemones always seem to respond by further relaxation. In addition, Panbin (1950) has noted that although food extracts often cause closed Metridium to open, there are times when the anemone remains closed. Since electrical stimulation of the SSl is also sometimes unsuccessful in causing a closed anemone to open, this suggests that the failure is probably not due to sensory adaptation or fatigue,

but to the fact that the retractors and sphincter remain contracted. During this study it was noted that closed anemones sometimes open slowly to SS1 stimulation, but close again immediately stimulation ceases.

There is no evidence so far to enable one to decide if the SS1 has a direct (or indirect) endodermal action, and it is not known whether the SS1 and SS2 are interconnected. McFarlane (1973a) suggested, however, that a link might exist between SS1 activity and spontaneous firing in the SS2 and nerve-net. It was noted that on the rare occasions when spontaneous activity in the SS1 was high there seemed to be a corresponding decrease in activity in the other two systems. This is a rather unusual response, since a reduction in the SS2 activity is normally succeeded by a burst of impulses in the through-conducting nerve-net. It may be that excitation of the SS1 can either directly or indirectly inhibit bursts of activity in the nerve-net and so modify the relationship between spontaneous nerve-net pulses and SP2s. If this is the case, then it is conceivable that impulses in the SS1 could cause relaxation in the endodermal muscles by inhibiting spontaneous firing in the nerve-net. This effect may be dependent on existing levels of spontaneous activity in both the SS2 and the nerve-net, and hence might explain the variable results obtained with closed anemones. It should be noted, however, that although excitation of the SS1 may bring about some sort of pacemaker inhibition in the endoderm, there is as yet no evidence for a connection between the SS1 and the endoderm.

The problem of conduction pathways between

excitable components of ectoderm and endoderm is also encountered in the shell-climbing response of Calliactis parasitica (McFarlane, 1969c) and in the swimming response of Stomphia coccinea (Robson, 1961c). In the latter case, sensory cells in the ectoderm must transmit excitation to the endodermal components responsible for the swimming activity, but there is no evidence that a pathway for such a link occurs in the column. It was proposed, however, that since the fibres of the parieto-basilar muscles are unusual in penetrating the pedal disc as far as the ectoderm, they could provide an anatomical pathway for nervous or sensory processes to pass from ectoderm to endoderm. Such pathways could conceivably link non-nervous systems like the SS1 and SS2. Another possible link may occur in the pharyngeal region. The nature of the epithelium lining the pharynx is uncertain, as it may be endoderm or modified ectoderm (Stephenson, 1928). It has been shown, however, that in Calliactis parasitica the boundary of the SS1 lies at the junction of the oral disc and pharynx (McFarlane, 1969b), and so the pathway by which the SS1 might modify activity in the endoderm remains unknown. The process by which a closed anemone opens in response to food could therefore involve either an additional sensory response, acting directly on endodermal elements, or a conduction pathway connecting the SS1 with the endoderm.

As mentioned earlier, this problem of endodermal involvement probably does not apply during relaxation of open anemones, due to the movement of the collar region, which twists round and down, thereby overcoming the problem of sphincter relaxation. These movements of the collar

Fig. 2.20. Showing movements of the collar region in Tealia during expansion and contraction. The photographs reveal that when the anemone is expanded (A) the collar is twisted in such a way that it points down towards the base of the column. As the anemone closes (B) the collar twists upwards, thereby enabling the sphincter to contract in the direction of its point of attachment to the body wall.

2.20

region can sometimes be observed quite clearly in certain specimens of Tealia. Fig. 2.20A is a photograph of such a specimen with a greatly distended capitulum. The collar region can be seen quite clearly, and the grooves above and below the collar are also evident. In this state of extreme expansion the collar is seen to be twisted downwards. In Fig. 2.20B the anemone is beginning to close up. The oral disc and tentacles are gradually withdrawing, owing to contraction of the radials and retractors, and the collar region has twisted upwards. This will then enable the sphincter to contract in the direction of its attachment to the body wall and hence shield the oral disc region from the exterior.

One further question that remains unanswered is this: what role might spontaneous contractions of the oral disc play in the intact animal? If the radial muscles were quiescent, then at rest they would assume a length dependent on those forces tending to stretch the oral disc. Stretching forces would be generated by such factors as a positive hydrostatic pressure in the coelenteron and the elasticity of the mesogloea. The function of the weakly-developed endodermal circular muscles in the oral disc is unknown, but this muscle layer is continuous with the circular muscle sheet of the column and could possibly govern tonic form in some way (Pantin, 1952). The internal hydrostatic pressure is determined mainly by endodermal muscle components in the column. The chief antagonists to the radials are presumably the column circulars acting through the pressure exerted on the coelenteric fluid. The mesogloea is reversibly extensible by very weak forces, although the rate of extension, even under large forces, is rather slow

(Batham & Pantin, 1950a). This property is also encountered in the mesogloea of the "solid-core" tentacles of Aurelias scyphistoma (Chapman, 1970). In this case, re-extension of the tentacles after contraction is believed to be achieved by viscoelastic forces in the mesogloea, although recovery is neither as fast nor as strong as might be expected with muscular antagonists.

As far as the oral disc is concerned, all of these factors will be tending to stretch the radials to their maximum extent. This would imply that if they were quiescent they would be incapable of relaxation, and hence unable to permit expansion of the oral disc. If this were the case, the only way in which the oral disc could expand would be by passive extension, due to an increase in internal hydrostatic pressure generated by contraction of the endodermal column circulars. Since the sensory information for the pre-feeding response is mediated by the SS1 (see Fig. 2.15, and McFarlane, 1970) it is clearly desirable that the ectodermal musculature should be responsible for expansion of the oral disc. In addition, involvement of endodermal muscles would be undesirable, as this would restrict such activity as columnar feeding movements.

The only way in which the radials could bring about expansion in an open anemone is by relaxation from an intermediate state of contraction. One way in which an intermediate tension may be maintained is to utilize the system employed in some crustacean muscle fibres. In these, the tension developed is closely related to the muscle membrane potential, and the strength of contraction is a function of impulse frequency in the motor nerve (Hoyle &

Wiersma, 1958). Hence, for such a muscle to maintain an intermediate state of contraction, the motor nerve would need to fire repetitively. Inhibition of this activity would then bring about relaxation of the muscle fibre. It has been shown that although the radials are innervated by the through-conducting nerve-net, no background electrical activity in the neurites supplying them can be recorded. Since such a system does not seem to apply to the radials, it is conceivable that intrinsic spontaneous activity in the muscles themselves could maintain an intermediate state of contraction. Inhibition of this activity, due to excitation of the SSl, would then result in expansion of the oral disc. The degree of expansion would be a function of the state of contraction of the radials acting against those forces tending to extend the oral disc.

The results obtained in this study seem to enforce many of the ideas propounded above, and have given us some insight into the mechanisms which control and coordinate oral disc activity in sea anemones. This section concludes the studies of effector activity in the pre-feeding response, and Part Three will deal with the sensory components involved.

SUMMARY OF PART TWO.

1. Radial muscle preparations from the oral disc of *Tealia felina* show periodical, spontaneous activity.

2. Electrical stimulation of the SS1 causes inhibition of spontaneous activity and increase in length of the radials. This response is elicited over a wide range of stimulus frequencies (from 1 shock every 2 sec to 1 every 60 sec). The response shows a slow onset and a long recovery period.

3. Stimulation of the nerve-net at frequencies between 1 shock every 5 sec and 1 every 20 sec produces slow contractions. The radials also show fast contractions to shocks less than 2 sec apart.

4. Dissolved food substances excite the SS1 in the column. The sensory response to application of food extract to a small area of the column shows evidence of sensory adaptation.

5. These observations are discussed in relation to the pre-feeding response of *Tealia* and a model for oral disc expansion is described. The mechanisms which control and coordinate activity in the oral disc are re-examined in the light of this new information.

PART THREE

CHEMORECEPTION IN THE PRE-FEEDING RESPONSE OF TEALIA FELINA

INTRODUCTION

In recent years much of the work on chemoreception in coelenterates has been concentrated on the identification of chemicals capable of evoking feeding responses. The first identification of a specific feeding activator was achieved by Loomis (1955) who found that the tripeptide reduced glutathione (GSH) could elicit a feeding response in Hydra littoralis. It was found to activate the response at concentrations as low as 10^{-6}M, and Lenhoff (1961) demonstrated that the response may last for upto 35 min. As Ross (1966) points out, this extensive period of activity might suggest that the response is due to a direct action of GSH on the muscle itself rather than on specific chemoreceptors. Evidence based on electrophysiological studies, however, is presented in this section to suggest that certain chemoreceptors in coelenterates may be unusual in that they remain active for long periods during chemical stimulation.

The demonstration that GSH was capable of evoking a feeding response in Hydra stimulated a great deal of interest in this field, and the feeding activators for a number of hydrozoans and anthozoans have since been identified. GSH acts as a feeding incitant in the hydroid Campanularia flexuosa (Lenhoff & Schneiderman, 1959), the siphonophores Physalia physalis (Lenhoff & Schneiderman, 1959) and Nanomia cara (Mackie & Boag, 1963), and the anthozoans Zoanthus sp. (Reimer, 1971a) and Diadumene luciae (Williams, 1972).

The heterocyclic amino acid proline activates the

feeding response in the hydroids Cordylophora lacustris (Fulton, 1963) and Pennaria tiarella (Pardy & Lenhoff, 1968), and in the sea anemone Calliactis polypus (Reimer, 1973). Either proline or GSH may act as feeding incitants in the Hawaiian corals Cyphastrea ocellina, Fungia scutaria and Pocillopora damicornis (Mariscal & Lenhoff, 1968), although proline appeared to be a more effective feeding activator than reduced glutathione, especially at low concentrations. Proline and GSH seem to have a synergistic effect as feeding activators in the tropical zoanthid, Palythoa psammophilia (Reimer, 1971b,c). In the sea anemone Anthopleura elegantissima the amino acid asparagine seems to initiate bending of the tentacles, and this results in food being transported across the oral disc and contacting the mouth (Lindstedt, 1971b). Once contact has been established, the tripeptide GSH activates the ingestion response. The amino acid valine acts as a feeding incitant in the swimming sea anemone Boloceroides sp. (Lindstedt, Muscarine & Lenhoff, 1968), and the amino acid arginine activates ingestion in the ceriantharian Pachycerianthus fimbriatus (Arai & Walder, 1973). Williams (1972) has shown that a number of amino acids and some vitamins of the B group are capable of activating the feeding response in the sea anemone Diadumene luciae, although GSH was found to be the most effective feeding activator.

All of these studies on feeding responses in coelenterates have used assays in which behavioural observations have been employed as the measured output of the sensory response. In no case has an attempt been made to obtain electrophysiological data in support of these

findings, and consequently it is difficult to determine whether one is observing a true chemosensory response, or merely the effects of specific chemical irritants acting directly on the muscles involved. Rushforth and Hoffman (1972) have recently made an attempt to correlate some electrophysiological observations with feeding behaviour in Hydra. They found that 10^{-5}M GSH had an inhibitory effect on Column and Tentacle Contraction Pulses, but the Rhythmic Potential Pacemaker System was unaffected. Unfortunately nothing that could be described as sensory activity was recorded, and the inhibitory effects of GSH may simply have been a direct chemical action on the muscles and conduction systems involved.

Although feeding responses can be elicited in some hydrozoans by chemical stimulation alone (Loomis, 1955; Lenhoff, 1961; Fulton, 1963), this does not seem to be true for sea anemones. Williams (1972) cites several examples in which a combination of mechanical and chemical stimuli are required to elicit feeding responses in sea anemones, and came to the conclusion that this situation probably applies to most macrophagous anthozoans. The pre-feeding response in sea anemones, however, is elicited by chemical stimulation, and is therefore, more amenable to electrophysiological analysis. McFarlane (1970) first demonstrated that preparatory feeding behaviour in Tealia felina involves excitation of the SSl, and McFarlane & Lawn (1972) have shown that this has an inhibitory effect on the radial muscles of the oral disc. Preparatory feeding activity, or the pre-feeding response, is elicited by dissolved food substances, and results in expansion of the oral disc

and lowering of its margin, followed by extension and "swaying" of the column. This behaviour clearly increases the food capture range of the tentacles, and enables the anemone to contact food which may be lying near the base of the column. It should, perhaps, be stated here that some workers engaged on research into feeding activity seem to be confused about the distinction between feeding and pre-feeding responses. Williams (1972) has assumed that as long as mechanical stimulation is absent a chemical stimulus producing any response when applied to any region of the anemone constitutes a pre-feeding activator. He therefore refers to chemicals which evoke contraction rather than extension of the tentacles as pre-feeding activators. This is, of course, the complete antithesis of a pre-feeding response which, by definition, involves extension of the tentacles and oral disc. A similar misunderstanding of the nature of pre-feeding activity appears in the study by Reimer (1973) in which preparatory feeding behaviour in Calliactis polypus is described as "tentacle writhing and twitching". Such activity would involve local asymmetric contractions of the tentacle longitudinal muscles, and this behaviour obviously cannot be related to the physiological activity associated with the pre-feeding response, as defined by McFarlane (1970), and McFarlane & Lawn (1972). Twitching, writhing and shortening of the tentacles should, I think, be designated as components of the feeding response, and consequently the "pre-feeding activators" described by these authors should rightly be considered as "feeding activators" capable of eliciting certain stages of the feeding response. A "pre-feeding activator" may be

provisionally defined as a soluble substance capable of eliciting expansion of the oral disc and extension of the tentacles by evoking activity in the SSl. To date, therefore, no pre-feeding activator has been identified.

The nature of chemoreception in coelenterates remains poorly understood, mainly because of the almost complete lack of electrophysiological and morphological data. The purpose of this study is to provide a relatively comprehensive account of the sensory component of the pre-feeding response in Tealia felina. Information derived from electrophysiological, biochemical, behavioural, and ultrastructural studies is presented here in the hope that it may throw some light onto our understanding of chemoreception in coelenterates.

MATERIALS AND METHODS

Specimens of Tealia felina were collected and maintained in the aquarium at the Gatty Marine Laboratory, as described in Part Two. Except where stated otherwise, the following experiments employed unoperated intact animals in order that the sensory responses could be studied under conditions approaching those that occur naturally. Both the common shore-form of Tealia felina (var. coriacea) and the deep-water form (var lofotensis) were used in these studies, as preliminary observations revealed no difference between the sensory responses of the two varieties. Only specimens in good condition with expanded oral disc diameters of 6-12 cm were selected for experiments, and these animals were then starved for 3 days before use. During experiments, specimens

were retained in running well-oxygenated sea water at temperatures ranging from 7-12°C. Electrical activity was recorded with suction electrodes, as previously described. Suction electrodes were also used for both electrical and chemical stimulation (see Part Two). The stimulating electrode used to deliver electrical shocks alone will be referred to as the ESE (electrical-stimulating electrode) and that used for both electrical stimulation and localized application of extract, and other substances, will be termed the CSE (chemical-stimulating electrode). The use of the CSE as a localized applicator of extract has been described in Part Two.

Preparation of Mytilus Extract: The mantle lobes and lamellae were dissected from living specimens of the bivalve mollusc Mytilus edulis and were homogenized in a small volume of sea water. The homogenate was then centrifuged at 10,000 rpm for 20 min at a temperature of 4°C, and the resulting supernatant used as the test extract. Extracts were stored at a temperature of 0°C, but were allowed to attain "sea-water temperature" before use in experiments. Freshly prepared extracts (less than 1 day old) were used in all experiments.

Column Chromatography: Fractionation of Mytilus extract was accomplished on a G-25 (medium) Sephadex column with a bed height of 60 cm. Such a column is capable of fractionating compounds with molecular weights ranging from 1000-5000. The extract used for column chromatography was prepared in the following manner: the mantle lobes and lamellae were removed from 3 Mytilus and washed in 10 changes of artificial sea water (see below) over a period of 30 min. The tissue was then homogenized in a small volume of artificial sea water

and centrifuged as previously described. Half of the volume of the resulting supernatant was retained without further treatment for control purposes. The remainder of the supernatant was fractionated on the chromatography column, and the eluted fractions were collected separately. From the calibration curve produced by chromatography of proteins of known molecular weight it was possible to estimate the range of molecular weight for each fraction collected. The ability of each fraction to activate the sensory response was then tested against the control extract.

Artificial Sea Water: The artificial sea water used in the fractionation experiments was made up according to the following formula (modified from Lyman & Fleming, 1940):

NaCl	23.5g
$MgCl_2$.....	5.0g
Na_2SO_4.....	3.9g
$CaCl_2$.....	1.1g
KCl.....	0.7g
$NaHCO_3$.....	0.2g
KBr.....	0.1g
Distilled Water.....	to 1000g

This formula includes all the major constituents of sea water and yields a solution of salinity 34.5‰, chloride content 19.0%, and pH 7.8. In practice this simple formula proved ideal for use in fractionation experiments.

Acetone-Treated Extracts: Aqueous extracts of Mytilus were treated with an excess of absolute acetone; the mixture was well shaken and then left at a temperature of -30°C for 30 min. The dielectric constant of the solution is decreased

by this treatment and this causes the proteins to precipitate out of solution. The mixture was centrifuged and the supernatant pipetted into a rotary evaporator. The acetone-soluble material was partially dried in the evaporator, redissolved in further absolute acetone, and recentrifuged. This procedure was repeated until no further precipitate was obtained with acetone treatment. The acetone-soluble material was then allowed to dry completely to ensure that all the volatile acetone and water components were driven off. The residue was then redissolved in artificial sea water and used as an acetone-treated Mytilus extract.

Electron Microscopy: Small pieces of tissue (less than 1 mm^3) taken from the ectodermal surface of the column of Tealia felina were fixed in 2.5% glutaraldehyde in 0.1M phosphate buffer and 0.375M NaCl at pH 7.4. The tissue was left in this medium for 10-12 hr at about $0^{\circ}C$, and then postfixed for 1 hr in 1% osmium tetroxide in 0.1M phosphate buffer and 0.375MNaCl at the same pH and temperature. The material was then dehydrated, at room temperature, through a graded series of ethanol, then treated with propylene oxide and finally embedded in Epon. Thin sections were cut with glass knives on an LKB ultramicrotome, stained with uranyl acetate and lead citrate, and examined in an A.E.I. EM6B electron microscope.

RESULTS

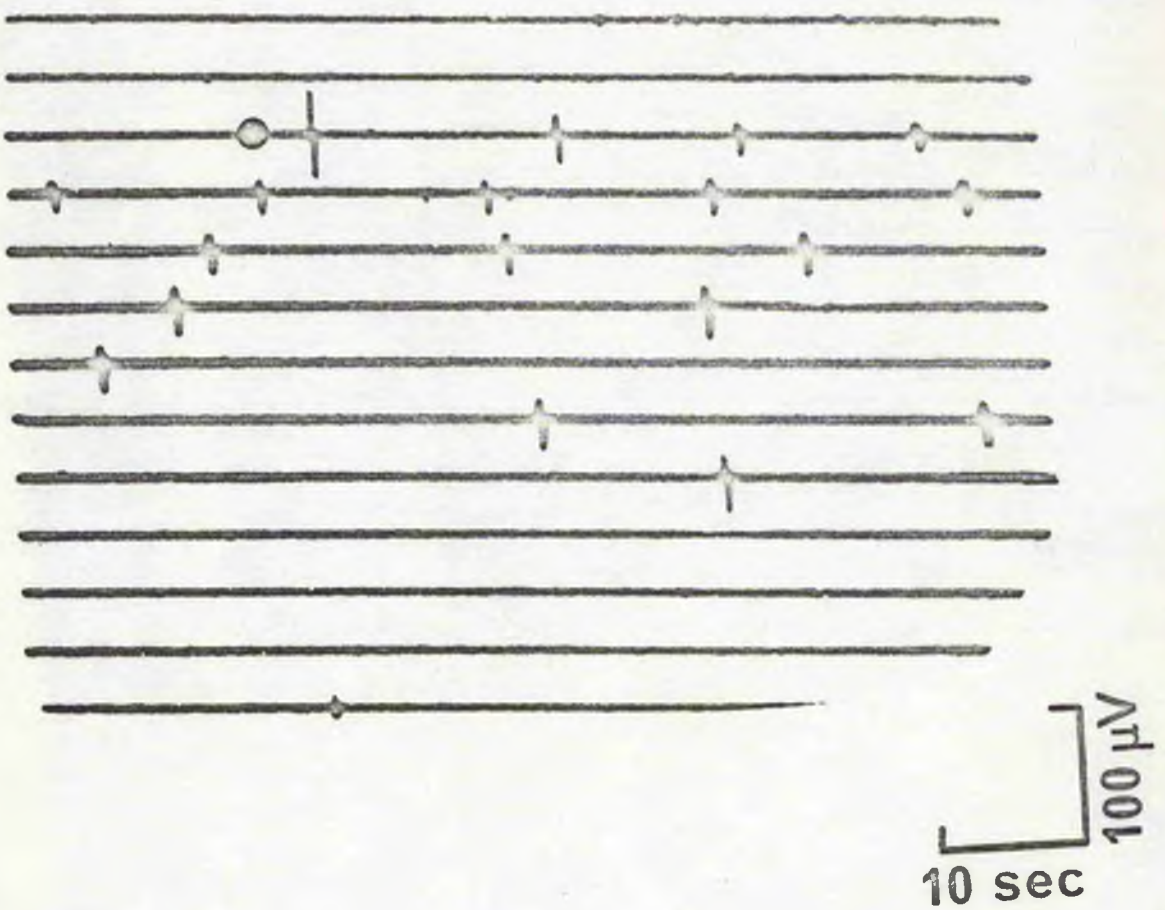
Sensory Adaptation

The placing of an electrode containing fresh Mytilus extract onto the column of Tealia felina normally

evokes SS1 activity and expansion of the oral disc (see Part Two). Control experiments with electrodes containing sea water alone indicate that the evoked SPIs are not a result of the applied suction. The sensory response seen in both radial muscle preparations (McFarlane & Lawn, 1972) and intact animals (McFarlane, 1970) seems to be identical. Fig. 3.1 is a recording of the sensory response obtained when dissolved food substances contact the column of an intact Tealia. This is the first direct recording of a chemosensory response in a sea anemone and shows that the frequency of evoked SPIs decreases during the response, even though an excess of stimulatory chemicals was provided. A graph demonstrating this phenomenon in a typical pre-feeding response is shown in Part Two (Fig. 2.16). Apart from possible sensory adaptation Fig. 3.1 reveals that the evoked SPIs tend to decrease in amplitude with increase in frequency. A similar phenomenon has been noted during repetitive electrical stimulation of the SS1 at frequencies down to 1 shock every 15 sec (see Part Two, Fig. 2.4). The reason for such a decrease in pulse size is unknown, but it is interesting to note that this effect can be reproduced during both spontaneous firing and electrical stimulation of the SS1. Unfortunately, it is not possible to determine whether these are genuine graded potentials until intracellular recordings can be obtained from the conducting elements involved. One can clearly see the relatively large amplitude of the initial SP1 and the gradual decrease in amplitude of successive SPIs during the first stage of the response. In the later stages, when the firing frequency decreases, there is a corresponding increase in pulse amplitude.

Fig. 3.1. Recording of electrical activity associated with the response to dissolved food substances. The dot denotes the application of extract to the column of Tealia. A number of SPIs are elicited as a result of this. There is a decrease in frequency of evoked SPIs as the response progresses. Note that as the frequency of evoked SPIs decreases there is a corresponding increase in pulse amplitude.

3.1



Occasionally, the sensory response to a single placement of the CSE may continue for considerably long periods, although there can be a great deal of variation in this respect. In some cases a placement will evoke only 1-2 SPls, or perhaps none at all, whereas in others single placements can evoke continual activity in the SSl for periods in excess of 1 hr. Such long duration firing appears to be a genuine response evoked by the food extract, for normally no spontaneous SPls are observed during 15 min monitoring periods prior to CSE attachment.

It was noted in Part Two that the decrease in frequency of evoked SPls during the sensory response may involve genuine sensory adaptation local to the point of stimulation, or may in fact involve fatigue in the SSl. In order to investigate this problem an electrical stimulating electrodes (ESE) was attached to the column of an intact Tealia and its ability to induce an SPl was tested by administering a shock at a voltage above SSl threshold. Spontaneous electrical activity in the intact animal was then monitored for 15 min, and during this time no spontaneous SPls were observed. The CSE containing fresh Mytilus extract was then applied to the column, about 1 cm from the attached ESE, and a typical sensory response was evoked. As the interval between successive SPls increased during the later stages of the response, single shocks were administered through the ESE. Each applied shock evoked a single SPl indicating that the SSl was not refractory between successive pulses in the later stages of the response. SPls could also be evoked by similar electrical or mechanical stimulation through the CSE, thereby indicating that the conducting

elements local to the chemoreceptive region were also in a non-refractory state. These observations suggest that the decrease in frequency of evoked SPIs during the pre-feeding response is due to sensory adaptation in the chemoreceptors involved. This sensory adaptation is remarkable in that it operates over a much extended time-scale compared with the same phenomenon encountered in higher animals.

Source of Evoked Electrical Activity

McFarlane (1970) has shown that the SSl in Tealia felina can also be excited by mechanical stimulation of the column, sensitivity being greatest near the base. If the column is prodded a nerve-net pulse usually accompanies the SPl, but a light touch with a clean brush often evokes one or two SPIs in the absence of nerve-net activity. Since mechanical stimulation, under certain conditions, is capable of evoking SSl activity, it remains possible that the food extract acts as an irritant and elicits local muscle contractions which pull against any points of attachment, such as pins in a preparation, or the substrate itself in an intact animal. It was, therefore, found necessary to determine the exact source of the evoked SPIs during the pre-feeding response, and for this reason two recording electrodes were employed.

A Tealia was bisected longitudinally without anaesthetic, and one half was placed column upwards onto a piece of cork and fixed down by four pins. The animal was left to recover for two days and appeared to be in good condition. The two recording electrodes (Rl and R2)

were attached to diametrically opposed tentacles, as shown in Fig. 3.2. For reference purposes, the pin through the lower column region on the same side as R1 was designated P1, and that on the opposite side P2. A simulating electrode (in this case electrical) is shown attached to the lower column adjacent to P1. The electrical events shown in Fig. 3.2 are simultaneous recordings from R1 and R2, and were evoked from a single shock applied through the ESE intthis position. Note that the SP1 arrives later at R2 than at R1 because of the longer conduction pathway between the point of stimulation and R2. This differential delay in arrival time at the two electrodes is less noticeable with the nerve-net pulse because of its higher conduction velocity.

This technique, therefore, may be used to determine the approximate source of evoked SPIs merely by comparing the simultaneous recordings from R1 and R2. Fig. 3.3 shows three such recordings in which single shocks were administered adjacent to P1 (Fig. 3.3.A) adjacent to P2 (Fig. 3.3.B) and finally at a point mid-way between P1 and P2 (Fig. 3.3.C). The recordings indicate that all the SPIs are evoked from the site of the stimulating electrode and not from some other, indirect source.

Similar experiments were carried out with a CSE containing Mytilus extract. In each case the electrode was applied to the column and an electrical shock administered through the CSE in order to determine the appropriate differential delay for this particular electrode placement. The SPIs evoked by the food extract were also monitored for this placement, and in all cases the differential

Fig. 3.2. Experimental arrangement to detect source of evoked electrical activity. The stimulating electrode was placed on the column close to the pin P1, as shown. The response to a single shock from this stimulating electrode is shown in the simultaneous recordings taken from R1 and R2. Note that both the nerve-net pulse (denoted by the dot) and the SP1 arrive later at R2 than at R1. This is due to the fact that the stimulating electrode is further from R2 than R1.

3.2

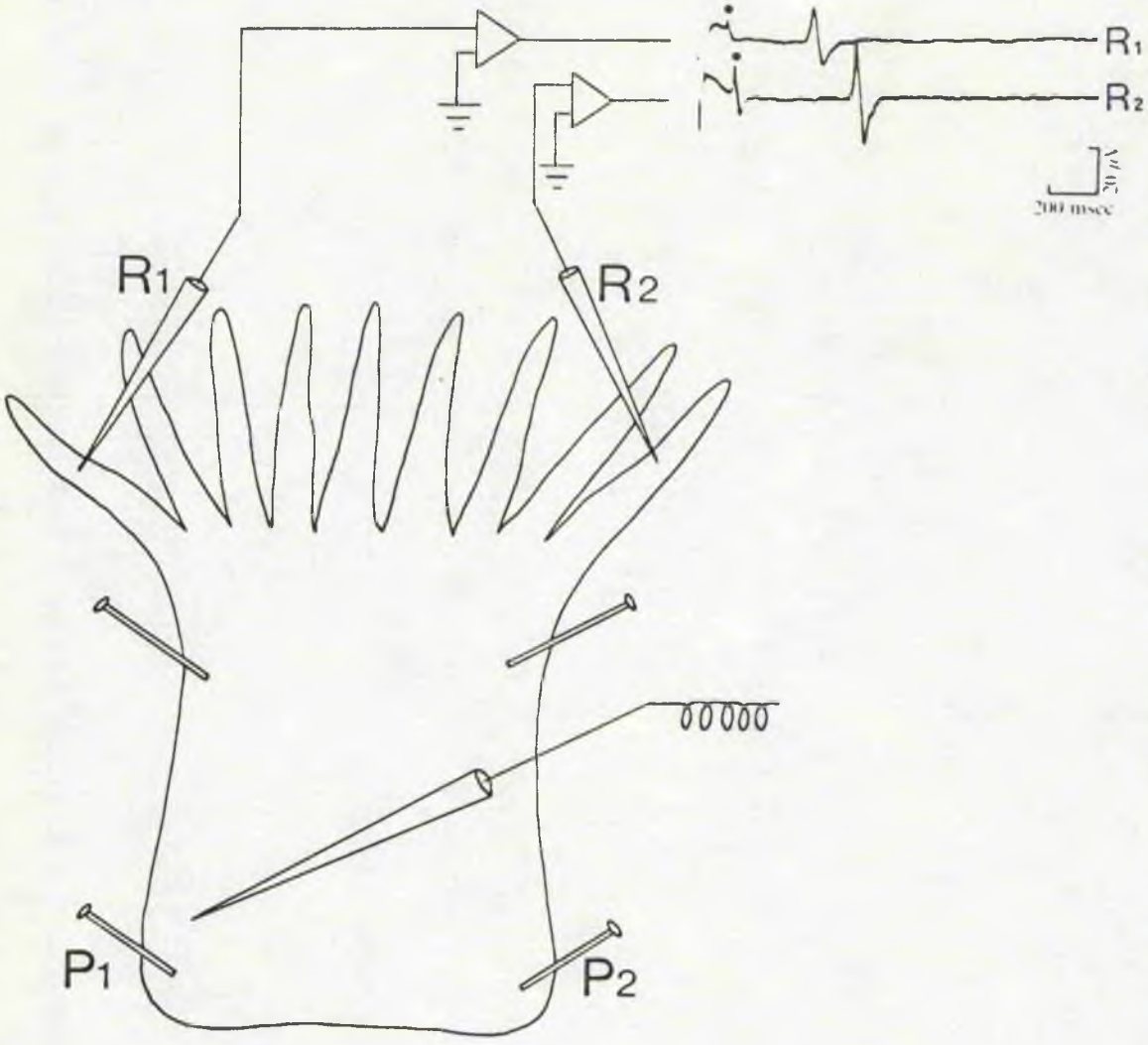
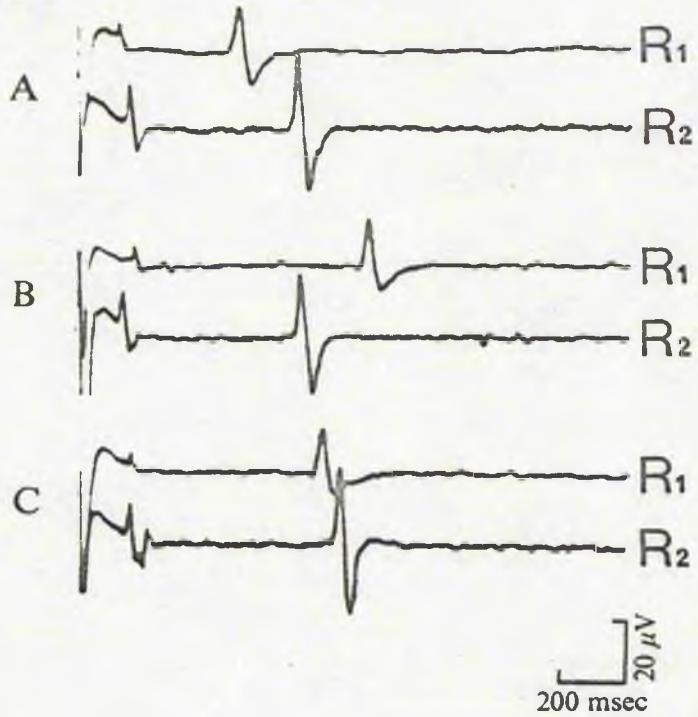


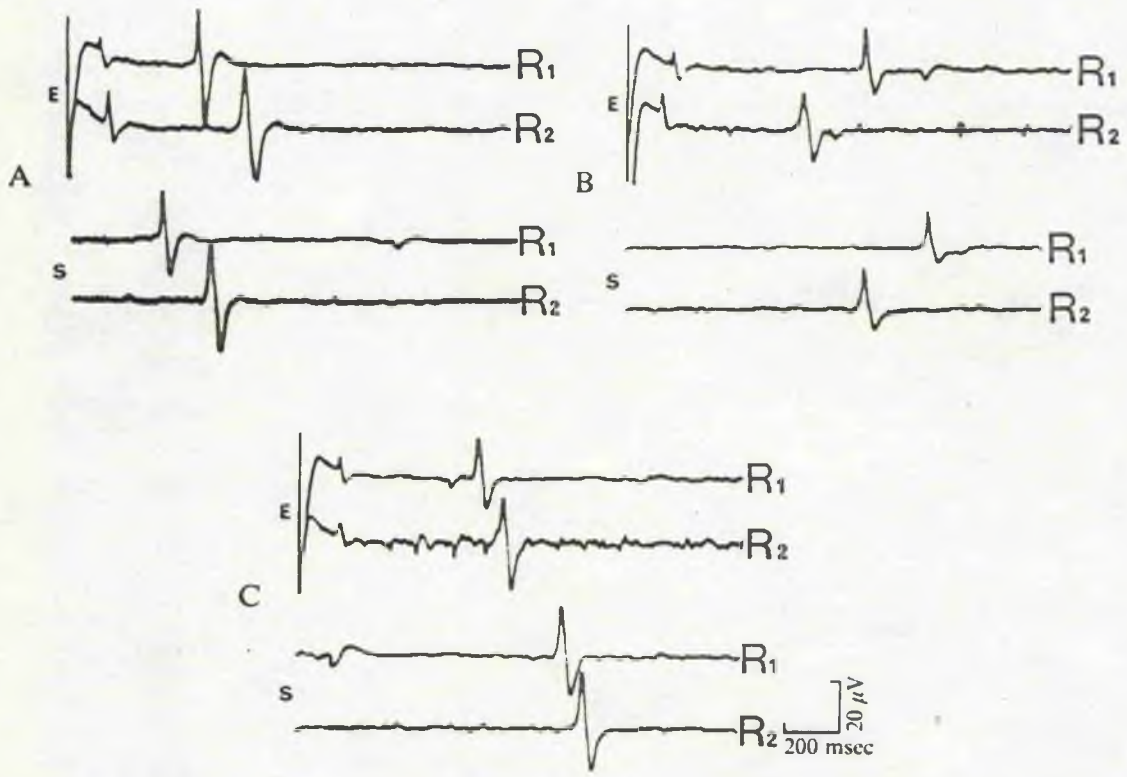
Fig. 3.3. Simultaneous recordings of electrical activity evoked by electrical stimulation at different points on the column. Experimental arrangement as shown in Fig. 3.2. Positions of stimulating electrode: (A) adjacent to P1, (B) adjacent to P2, (C) mid-way between P1 and P2. The recordings demonstrate that this technique may be employed to pinpoint the source of evoked electrical activity.

Fig. 3.4. Source of SPIs evoked during the sensory response to dissolved food substances. A chemical-stimulating electrode (CSE) containing food extract was applied to different points on the column. In each case a single electrical shock was administered through the electrode (upper two traces in each recording - marked E) to determine the differential delay in R1 and R2 for particular placements. The SPIs evoked during the sensory response to the food extract were also monitored (lower two traces in each recording - marked S) to compare their differential delays with those of the electrically evoked SPIs. Positions of CSE: (A) adjacent to P1, (B) adjacent to P2, (C) mid-way between P1 and P2. These recordings confirm that the SPIs evoked during the sensory response originate from the site of of the applied chemical stimulus.

3.3



3.4



delays were identical to those obtained with electrical stimulation (see Fig. 3.4). This would indicate that all the evoked SPIs in the sensory response originate from the site of the applied chemical stimulus and are not elicited by some form of stimulation at a region remote from this site.

Location of Receptor Sites

Once it had been established that the sensory response was in fact elicited from a chemoreceptive region contacting the locally applied food extract it was possible to use this technique to delineate the extent of the chemoreceptors involved in the pre-feeding response. The CSE containing fresh Mytilus extract was applied to various regions of the column, pedal disc and pharynx in order to define those regions capable of eliciting the chemosensory response.

Intact animals and half-animal preparations were employed in these experiments, and several different specimens of Tealia were used to overcome the problem of individual variation between animals. The various regions tested are depicted in Fig. 3.5. For the purposes of this study the column (more specifically the scapus) has been subdivided into a number of regions. The collar region is that portion of the column enclosing the sphincter. Immediately below the collar is the upper column, and below this is a more extensive mid-column region in which the majority of the warts (or verrucae) are concentrated. The region below this contains a lower density of verrucae and is termed the

lower column, and below this is a region of column which eventually connects with the margin of the pedal disc.This is termed the column base and the complete absence of verrucae in this region distinguishes it from the lower column. It should be stressed that such anarbitrary sub-division of the column is merely for the purposes of this investigation, and does not necessarily imply that a functional or structural differentiation exists between these regions.

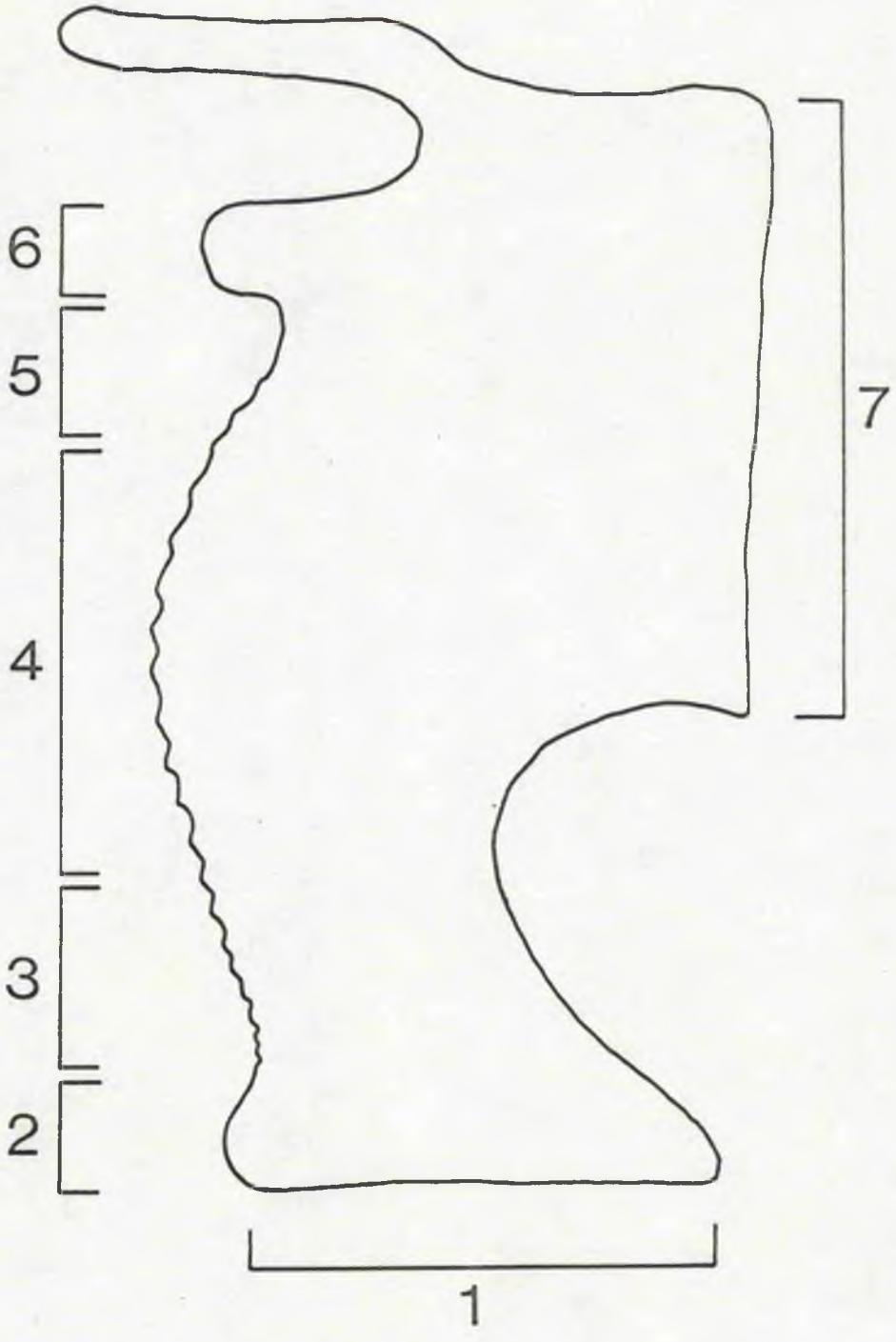
The results of a typical experiment are shown in Table 3.1. The data suggest that a sensory response can be obtained from all parts of the column, although it was consistently found that the upper column and collar regions were lass responsive than the mid-column, lower column and column base. The pedal disc appeared to give no sensory response to food extract. The two SPls recorded in Table 3.1 were probably due to mechanical stimulation as it was noted that the detached pedal disc was particularly sensitive in this respect. The pharynx also appeared to be incapable of evoking SPls in response to dissolved food substances. The ability of the pharynx to conduct SPls was confirmed by applying a suitable electrical stimulus through the CSE. It remained possible that mucus from the pharynx might be blocking the tip of the electrode and hence preventing food extract from contacting the pharyngeal epithelium. To investigate this possibility the CSE was applied to the pharynx and left attached for 5 min. No SPls were monitored during this period. The CSE was then gently detached from the pharynx with a minimum of disturbance to the electrode and its contents, and immediately placed on the mid-column

Table 3.1. Results taken from a typical experiment to determine the responsiveness of different regions of Tealia to dissolved food substances. Regions tested are depicted in Fig. 3.5. Data obtained from a total of 4 placements with the CSE have been tabulated.

REGION TESTED	NUMBER SPIs EVOKED IN 5 MINUTES (4 PLACEMENTS PER REGION)				TOTAL SPIs IN 4 PLACEMENTS
Pedal Disc	0	0	2	0	2
Column Base	6	8	4	5	23
Lower Column	3	5	5	6	19
Mid-Column	9	3	6	4	22
Upper Column	4	0	5	5	14
Collar Region	3	5	0	1	9
Pharynx	0	0	0	0	0

Fig. 3.5. Regions tested to determine the location of chemoreceptor sites. The diagram represents one side of a bisected Tealia. The regions tested were: 1, pedal disc; 2, column base; 3, lower column; 4, mid-column; 5, upper column; 6, collar region; 7, pharynx.

3.5



region of the animal. Within the first two minutes of placement 5 SPIs were monitored confirming that any mucus on the tip of the electrode was not preventing contact of the food extract with the chemoreceptors. Suitable controls, using sea water in place of food extract, were interspersed throughout these experiments.

The results, therefore, seem to indicate that the chemoreceptors responsible for the sensory component of the pre-feeding response are dispersed throughout the column ectoderm of Tealia, with possibly a denser concentration of receptors in the middle and lower regions. It was noted during these experiments that not every column placement evoked a sensory response and this may indicate that the chemoreceptors are locally concentrated within the responsive regions. It is difficult to confirm this observation conclusively, however, because of the variable nature of the response for each placement, even in the same region.

The pre-feeding chemoreceptors seem to be entirely absent from the pedal disc and pharynx, even though these regions are capable of conducting SPIs. It was found impossible to test the responsiveness of the oral disc and tentacles to food extract because this evoked true feeding responses, involving complex electrical activity which entirely obliterates any recordings of the sensory responses. This activity is also accompanied by local contractions of the tentacles which tend to pull against the suction electrodes until they eventually become detached.

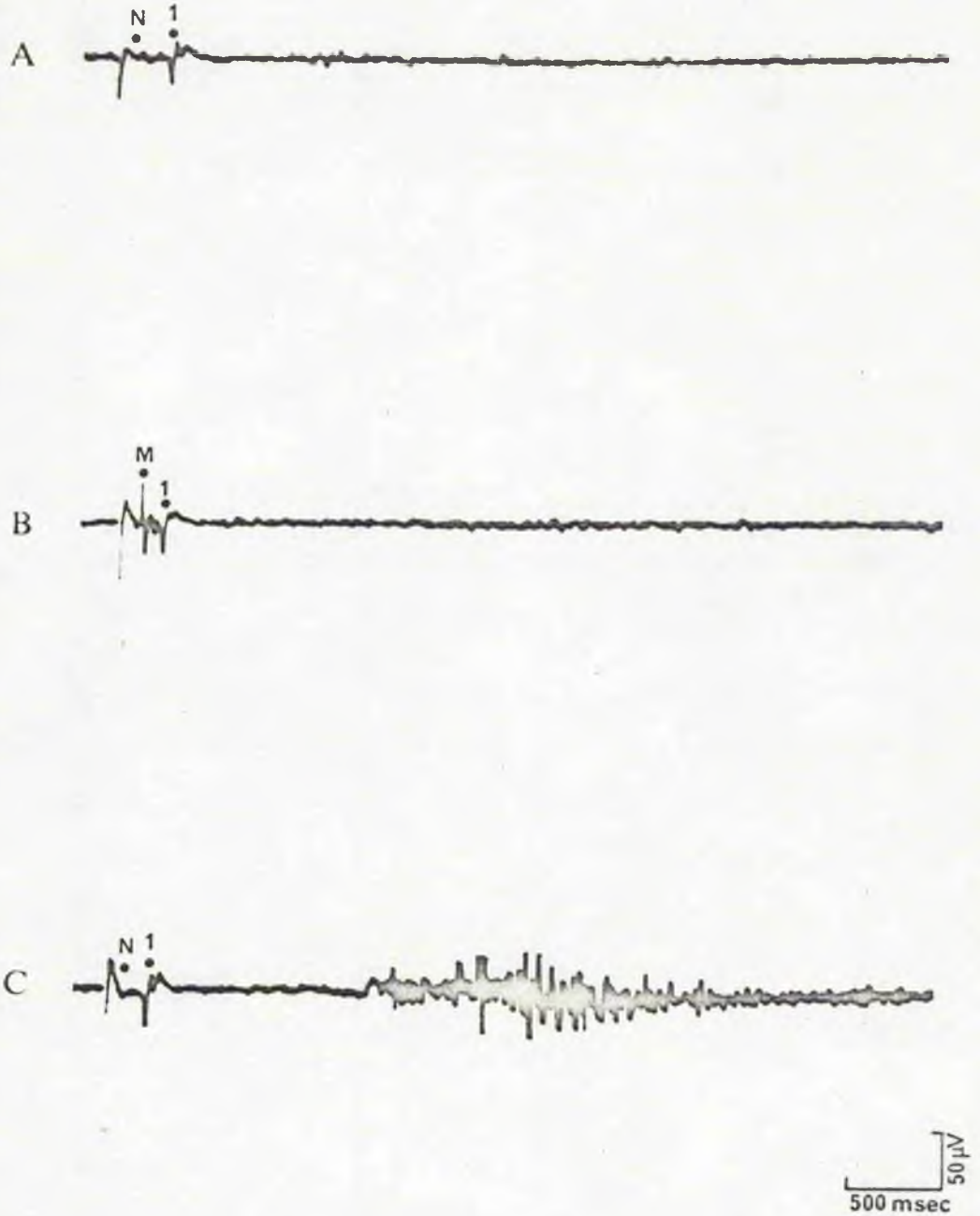
Little is known about the nature of complex activity and its role in the neuromuscular physiology of the anemone. It can be evoked by electrical stimulation

of the oral disc or tentacles, and this proves to be a more convenient means of studying it, for the recording electrode often remains attached if stimulation is not excessive. Fig. 3.6 shows some typical responses to electrical stimulation of a tentacle 2 cm away from the tentacle attached to the recording electrode. Fig. 3.6A shows the electrical activity evoked by a single shock at a stimulus intensity of 2.5V. Note the small pulse associated with the nerve-net, the single SP1, and the absence of any other electrical events. If the stimulus intensity is increased the tentacle sometimes (but not inevitably) gives a twitch to a single shock. Such a response is seen in Fig. 3.6B where a muscle action potential in the tentacle is seen immediately following the through-conducted nerve-net pulse. This activity was accompanied by a twitch of the tentacle concerned, but no complex electrical activity was evoked. The SP1 is seen to follow the muscle action potential after the appropriate conduction delay. Fig. 3.6C shows the effect of stimulating at a relatively high intensity (10V). A single nerve-net pulse (in this case without an accompanying muscle action potential) and an SP1 are evoked. There then follows a short quiescent period before a sequence of complex activity ensues.

As this activity does not seem to be accompanied by excitation of the nerve-net (as with the muscle action potential in Fig. 3.6B) it is possible that these graded potentials represent local muscular activity propagated through the muscle field itself. Such a mechanism has been proposed by McFarlane & Lawn (1972) (see Part Two) to explain

Fig. 3.6. Activity evoked in response to electrical stimulation of a tentacle. The stimulating electrode was attached to a tentacle only 2 cm away from the tentacle bearing the recording electrode. Intensity of stimulation: (A) 2.5V, (B) 5V, (C) 10V. In each case a single shock was applied. In A there is a very small nerve-net pulse (N) followed only by a single SP1(1). A musole action potential (M) immediately follows the nerve-net pulse in B, but otherwise the electrical activity is essentially the same as that recorded in A. In C a nerve-net pulse and an SP1 are evoked in response to the stimulus. A quiet period then follows before a sequence of complex electrical activity ensues. It is possible that such activity consists of graded potentials associated with local muscular activity.

3.6



local activity in the oral disc radials and tentacle longitudinals. If one assumes that the direct conduction pathway from the stimulating electrode to the recording electrode is 4 cm (1 cm along each tentacle and 2 cm across the oral disc) then the records indicate that the conduction velocity of the SS1 in the oral disc is about 20 cm/sec, that of the nerve-net about 60 cm/sec, and that of the muscular activity about 3.5 cm/sec at a temperature of 9°C. The intensity and duration of the sequence of complex activity seems to increase with the intensity of the applied stimulus, and this would also suggest that graded local responses are involved. The relatively low conduction velocity of the complex activity corresponds with the observation that local contractions are also conducted at similar velocities. Obviously, this local activity in the tentacles and oral disc plays an important role in the transfer of food to the mouth during the feeding response.

Identification of the Pre-feeding Activator

The sensory response evoked by the application of dissolved food substances to the column of Tealia provides a clearly defined bioassay for the identification of active compounds. Assays using electrophysiological techniques allow the observer to express the results in precise quantitative terms, unlike the assays presently employed to study feeding activity in coelenterates (see review by Lindstedt, 1971a) which rely on purely behavioural observations as the measured output of the response.

In the following experiments the solutions being tested were applied to the column of Tealia with a CSE and the presence or absence of an evoked sensory response was noted. For every test solution a minimum of 5 placements on different regions of the column were undertaken to ensure that the observed response was genuine and repeatable. Stringent controls were interspersed throughout these experiments, utilizing both Mytilus extract to give a positive sensory response, and sea water to give a negative response. Only results from those experiments giving good controls and repeatable test responses were regarded as genuine, thereby minimizing the problem of variation between individual placements.

1) Responses to Mytilus extract: Mytilus extract was treated in various ways in order to determine the nature of the active component. Heating the extract for 1 hr in a boiling water bath did not impair its ability to evoke SPIs when applied to the column of Tealia. Heating is usually assumed to denature proteins in solution, but recently Gurin & Carr (1971) have shown that certain stimulatory proteins capable of inducing a strong feeding response in the marine snail Nassarius obsoletus are not deactivated by heating in a water bath. If the Mytilus extract is treated more drastically by boiling to dryness and then redissolving the residue in artificial sea water it is still capable of evoking the sensory response in Tealia. This confirms that the active component is heat stable, and would suggest that it is non-proteinaceous.

Treatment of extract with acetone precipitates proteins out of solution. The acetone-soluble residue,

redissolved in artificial sea water, evoked a strong chemosensory response, and this would again suggest that the active component is non-proteinaceous. Fractionation of Mytilus extract by column chromatography yielded several fractions containing compounds of known molecular weight. All fractions with a molecular weight above 1000 gave negative sensory responses, whereas the fraction containing compounds with molecular weights of less than 1000 gave a positive response. Unfortunately, it proved impossible to subdivide this fraction further by column-chromatographic techniques. The fact that the active compound has a molecular weight of less than 1000 would again suggest a non-proteinaceous compound.

2) Analysis of active fraction: The carbohydrate content of the active fraction was analysed by gas/liquid chromatography using a hydrogen-flame ionization detector. The fraction was dried in a rotary evaporator and the residue dissolved in pyridine. Chromatographic separation at a constant temperature of 140°C revealed the presence of a peak on the chromatogram corresponding to the hexitol mannitol (mol. wt. 182.18). Monitoring of the chromatogram was continued for 30 min and no further peaks were detected in this period. Separation at a temperature of 100°C revealed the presence of the trihydroxyl alcohol glycerol (mol. wt. 60.05). This low-temperature separation serves to isolate the glycerol peak on the chromatogram from that of the solvent peak (pyridine). In summary, therefore, gas/liquid chromatography reveals the presence of two carbohydrates in the active fraction— glycerol and mannitol.

Analysis of the amino acid content of the active

fraction was achieved by microchromatography of dansylated compounds. The residue of the dried fraction was dissolved in 70% acetone and this solution was chromatographed on 3x3 cm polyamide layers. The developed chromatograms were dried carefully and then placed in a sealed jar containing paraformaldehyde. The jar was heated in an oven for 3 hr at a temperature of 80°C and the amino acids were then located by viewing the chromatograms under UV-light (for details of technique see Osborne, 1971).

This method allows one to make a qualitative and semi-quantitative analysis of the amino acid content of the active fraction. An attempt has been made to denote the relative quantity of each amino acid in the fraction by a simple plus-minus system. The absence of a symbol denotes a standard concentration, a single minus a relatively low concentration, a single plus a slightly higher than standard concentration, two pluses a high concentration, and three pluses a very high concentration. The following amino acids were found to be present in the active fraction:

1) Alanine ..... ++
2) Isoleucine .....
3) Leucine.....
4) Lysine..... +
5) Methionine..... -
6) Ornithine..... -
7) Phenylalanine.....
8) Proline..... ++
9) Taurine..... +++
10) Tryptophan.....
11) Valine.....
12) Others (unidentified)

3) Tests with identified compounds: An attempt was made

to discover the individual compound (or compounds) responsible for activating the sensory component of the pre-feeding response. The previous experiments had established that the pre-feeding activator has a molecular weight of less than 1000, is heat stable, soluble in absolute acetone, and therefore probably non-proteinaceous. A wide range of compounds fulfilling these requirements were assayed to establish their potency in evoking the sensory response. Compounds were dissolved in artificial sea water buffered at pH 7.0 (the same pH as that of the active fraction) to ensure that the response would not be evoked by a change of pH. The active fraction and the buffered artificial sea water were used as controls between assays of individual solutions. Solutions at a concentration of 10^{-2}M were used throughout, for higher concentrations of individual compounds are unlikely to be encountered under natural conditions. It was found that 1 ml of the active fraction still retained its potency after dilution in 100 ml of artificial sea water. Sensitivity to such low concentrations of dissolved food substances might be expected in a pre-feeding response.

The compounds that were assayed during this investigation are listed in Table 3.2 and all were found to be incapable of evoking the sensory component of the pre-feeding response. Several common amino acids were assayed in addition to those detected in the active fraction. The non-ionic surfactant Triton X-100 was also tested, as the detergent or surface-active properties of this polymer seem to evoke the avoidance reaction in molluscs by damaging the chemoreceptors involved (Mackie, Lasker & Grant, 1968). This also gave a negative response. Various

Table 3.2. Compounds assayed in an attempt to identify the activator of the pre-feeding response. All were found to be incapable of evoking the sensory component of the response.

INDIVIDUAL COMPOUNDS TESTED (ALL NEGATIVE)	
Carbohydrates:	Glycerol
	Mannitol
Amino acids and related nitrogenous compounds:	Alanine
	Arginine
	Asparagine
	Glutamic acid
	Glutamine
	Glutathione (reduced)
	Glycine
	Isoleucine
	Leucine
	Lysine
	Methionine
	Ornithine
	Phenylalanine
	Proline
	Serine
	Taurine
	Tryptophan
	Tyrosine
	Valine
Others:	Triton X-100

MIXTURES TESTED. (ALL NEGATIVE)
Bactopeptone
*Casamino Acid
Yeast Extract (Marmite)
General Mixture

*Constituents of Casamino Acid

Amino acids and related compounds

Arginine
Aspartic Acid
Choline
Cystine
Glutamic Acid
Glycine
Histidine
Isoleucine
Leucine
Lysine
Methionine
Phenylalanine
Proline
Threonine
Tryptophan
Tyrosine
Valine

Vitamins

Biotin
Cyanocobalamin
Folic Acid
Niacin
Pantothenic Acid
Pyridoxin
Riboflavin
Thiamine

mixtures were assayed to establish whether the sensory response could be evoked by a combination of different compounds. Bactopeptone and casamino acid are sources of several compounds. The constituents of casamino acid are listed in Table 3.2, and it can be seen that a wide range of amino acids and vitamins are represented. Yeast extract (Marmite) was also tested as this is a source of several B vitamins. Finally, a general mixture was prepared from all the compounds previously assayed in this study, and this also produced a negative response.

It is, perhaps, unfortunate that insufficient time was available to test further amino acids, vitamins and otherocompounds. It was possible, however, to establish that a positive sensory response could be obtained from an acetone-soluble extract taken from the terrestrial gastropod, Helix aspersa. This would suggest that the active compound is not exclusive to marine molluscs and is probably widely distributed throughout the animal kingdom. A more detailed biochemical analysis of the fraction, and further assays employing a wide range of organic (and perhaps inorganic) compounds should eventually lead to the identification of the pre-feeding activator. Until this hasbbeen achieved one can only describe it as a heat-stable, acetone-soluble compound with a molecular weight of less than 1000.

Structural Studies on the Column of Tealia felina

A preliminary study on the fine structure of the column ectoderm of Tealia was undertaken to provide morphological evidence for the existence of chemoreceptors in

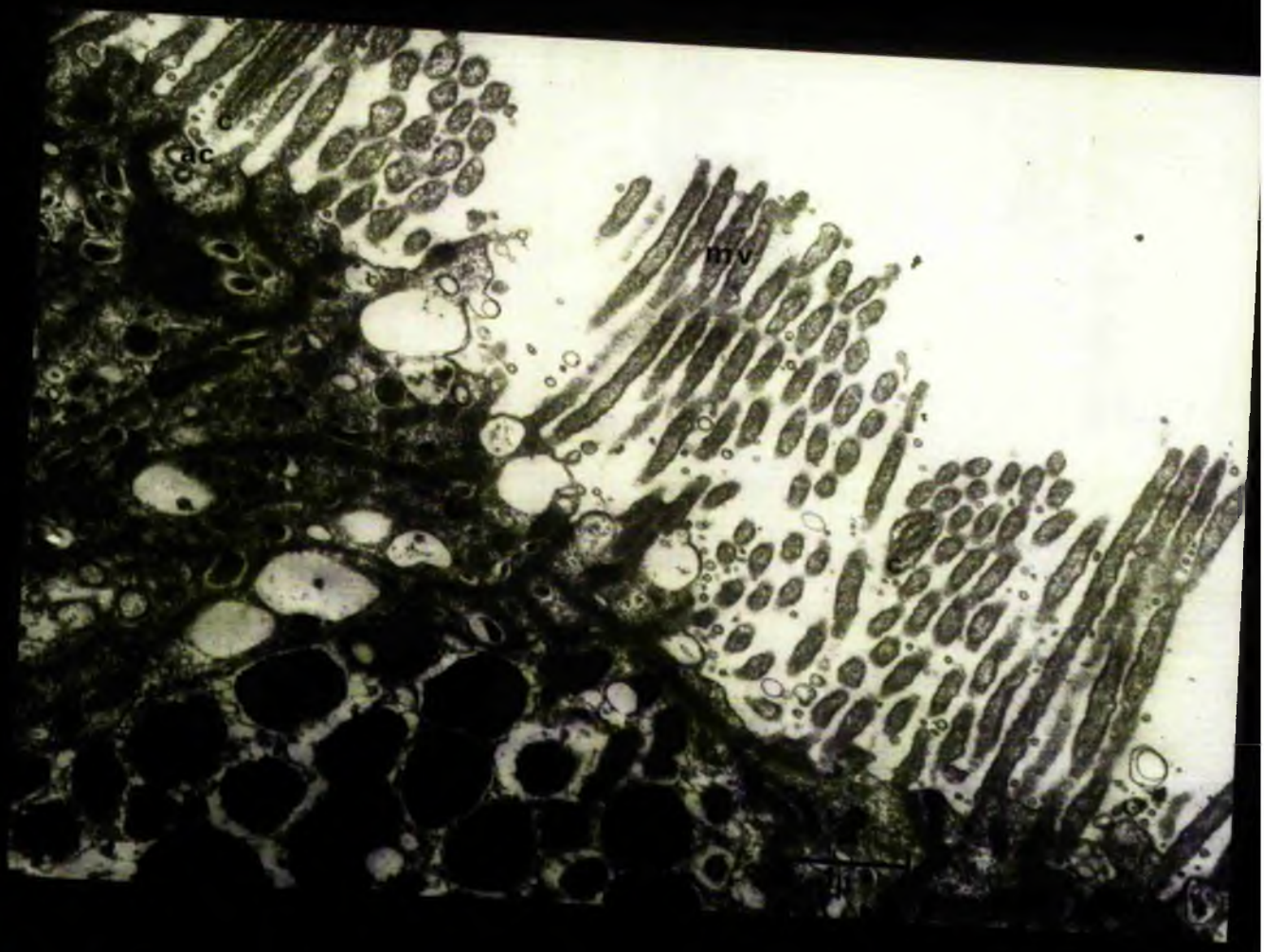
this region. Fig. 3.7 is an electronmicrograph showing a typical region of the ectodermal surface of the column. The most striking feature is the presence of large numbers of microvilli projecting from the free surface of each epithelial cell. These microvilli seem to be longer, more variable in diameter, and more disorderly than those comprising the brush borders on renal and intestinal epithelia of vertebrates, and probably correspond to the "stereocilia" described by light microscopists. This resemblance to non-motile cilia disappears at the level of resolution of the electron microscope for these cell processes have no trace of the complex internal structure so characteristic of cilia. It is generally accepted that the function of microvilli is to effectively extend the surface area of the cell membrane and thereby permit an overall increase in the rate of diffusion of substances passing into and out of the cell. As far as the column of Tealia is concerned these microvilli may have a respiratory, and perhaps excretory, function as they would be in permanent contact with the surrounding sea water. Similar microvilli on the free surface of the gastrodermis are probably responsible for the absorption of certain food substances during the digestive process.

The possibility remains that the microvilli of the columnar epithelium may have a chemosensory function. Ultrastructural studies on the taste buds of the rabbit (de Lorenzo, 1958) reveal that the apical tips of the receptor cells are characterised by the presence of numerous microvilli. In addition, the vomeronasal receptor cells in many vertebrates possess microvilli which are believed to function as chemo-

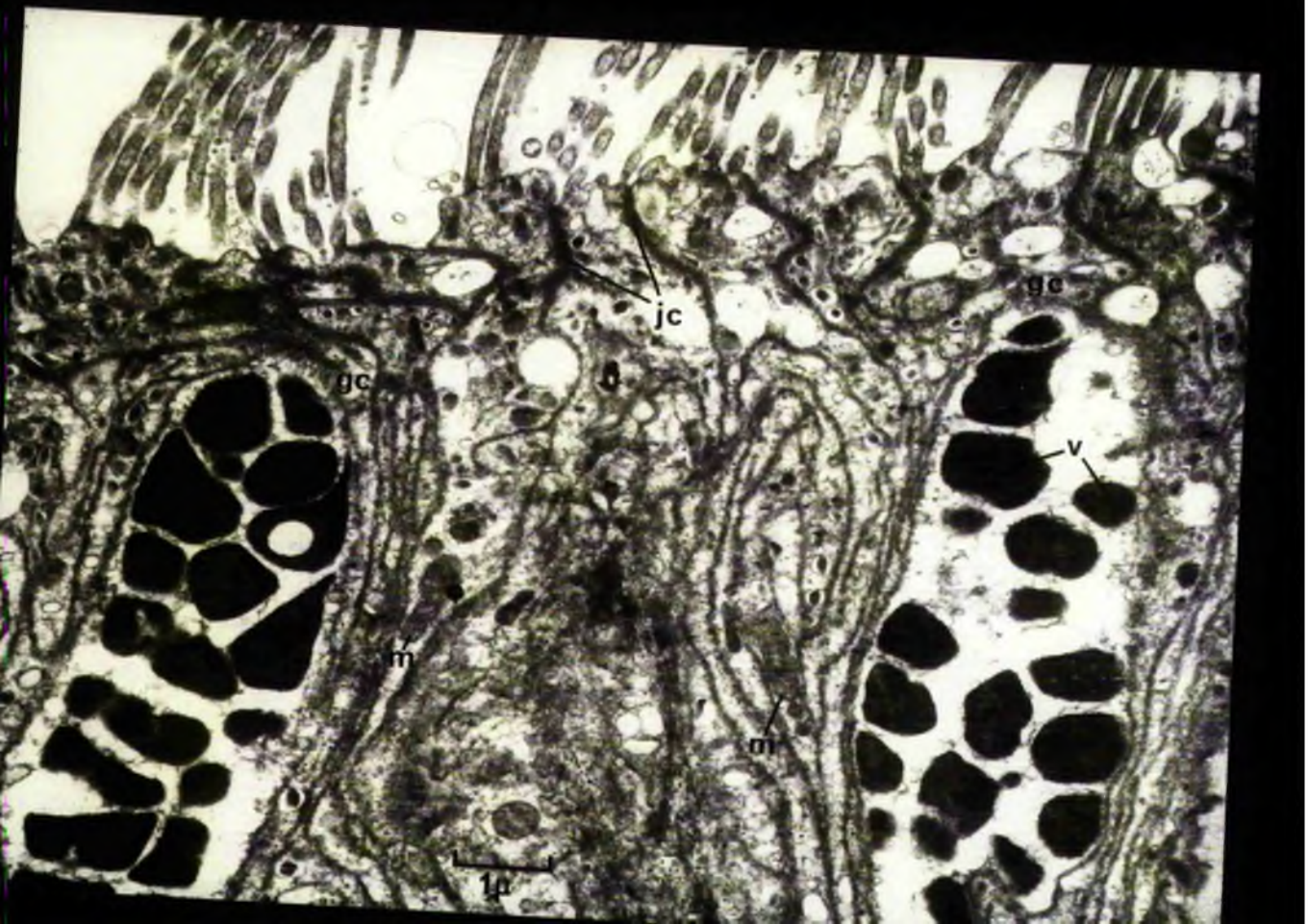
Fig. 3.7. Electron micrograph showing a region of the ectodermal surface of the column of Tealia. The section was cut at right angles to the plane of the column surface. Note the presence of cilia (c) and the large numbers of microvilli (mv) projecting from the free surfaces of the epithelial cells. Note that the apex of the ciliated cell in the upper left-hand corner of the micrograph is indented in the region of the cilium to form an apical collar (ac). It is suggested that the cilia may function as chemoreceptors involved in the pre-feeding response.

Fig. 3.8. Electron micrograph of another region of the ectodermal surface of the column. Note the large electron-dense vesicles (v) of the gland cells (gc) and the electron-dense junctional complexes (jc) which exist between opposing membranes of the epithelial cells. Mitochondria (m) can also be identified. No structures that could be positively identified as nerve processes were observed in the column ectoderm.

3.7



3.8



sensory structures. A study of the development of the vomeronasal receptor cells in mice (Bannister & Cuschieri, 1972) has revealed that the receptive surface area is increased by the formation of microvilli. It is clearly possible, therefore, that the microvilli on the columnar surface of Tealia may also function as chemoreceptors.

Cilia are also encountered on the column of Tealia, although they are more widely dispersed and less numerous than the microvilli. Two cilia are seen in Fig. 3.7 where one is sectioned obliquely and the other (in the top left-hand corner) longitudinally along the stalk of the cilium. The electronmicrograph gives an indication of the relative numbers of cilia and microvilli encountered over the entire columnar epithelium. All the cilia observed during this study invariably possessed the typical 9 peripheral pairs and 2 central filaments in the ciliary stalk, and these were accompanied by basal bodies and ciliary rootlets below the level of the stalk. There is some evidence to suggest that these cilia are non-motile, or at least restricted in their movements. The fact that they are completely surrounded by long densely-packed microvilli would suggest that any movement of the cilium would be severely restricted. In addition, direct observations of freshly-dissected pieces of column placed in a drop of sea water on a glass slide and viewed under the highest magnification (X1,700) of the phase-contrast microscope, revealed that the cilia, which project above the surrounding microvilli, are stationary. Similar observations on cilia lining the surface of freshly dissected pieces of tentacle taken from Calliactis parasitica revealed that these cilia

remained highly motile for upto 1 hr after sectioning. This would suggest that the dissection process does not inhibit ciliary activity in freshly-sectioned material, and implies that the cilia observed on the column of Tealia may be genuinely non-motile. It is, therefore, highly probable that these cilia function as receptors of some sort, conveying sensory information into the ectodermal epithelium.

Cilia are believed to have a receptive function in a wide range of sense organs in many different groups of animals. In vertebrates the olfactory epithelial cells are provided with one or several cilia, and cilia are also found in olfactory cells of insects (Slifer, 1961) and in marine and terrestrial decapods from a variety of habits (Ghiradella, Case & Cronshaw, 1968a,b; Frisch & Everingham, 1972). In the olfactory epithelium of the rabbit 6 to 12 cilia are seen projecting from each receptor cell (de Lorenzo, 1963), and these have a typical ciliary structure. These cilia always seem to be enmeshed in microvilli and it has been suggested that a functional relationship may exist between these structures. Focal dilations of the cilia are commonly found in olfactory organs and Frisch & Everingham (1972) suggest that these correspond to localized permeability changes in the ciliary membrane associated with sites of chemoreception. No focal dilations were observed in the cilia of the columnar epithelium of Tealia, but this does not, in itself, preclude a chemoreceptive function for these cilia. As Lettvin and Gesteland (1965) point out "There is an impressive ignorance of sensory cilia wherever they occur". Lentz & Barrnett (1965) describe possible sensory cells in Hydra which seem to correspond closely with the columnar cilia of Tealia. In Hydra

the "sensory cells" are interspersed between the apices of surrounding cells in the epidermis and gastrodermis, and the apex is indented in the region of the cilium to form an apical collar. A similar structure is seen at the apex of the ciliated cells in the column of Tealia, as denoted in Fig. 3.7. The ciliated cells in Hydra were tentatively assigned a photoreceptive function, but the evidence for this speculation was entirely circumstantial. As no photopigment was recognized, and as the photoreceptor in the hydromedusan Polyorchis penicillatus has a more complex structure (Eakin & Westfall, 1962) it is possible that these cilia may function as chemoreceptors or mechanoreceptors, or perhaps both. The flagella of the nematocysts of sea anemones may function both as chemoreceptors and mechanoreceptors (estfall, 1965), but as Ross (1966) points out a more economical arrangement would involve a single set of chemoreceptors initiating the neuromuscular response of the tentacles, and also sensitizing the cnidoblasts to mechanical stimulation. In this context one must also bear in mind that spirocysts, which have no flagella directly associated with them, are sensitive to specific chemical stimulation.

McFarlane (1970) has shown that the SSl in Tealia can be excited by both chemical and mechanical stimulation. It is obviously impossible at this stage, therefore, to determine whether the column cilia are mechanoreceptors or chemoreceptors. The physiological evidence presented in this section suggests that the chemoreceptors may be widely separated, as a localized application of food extract to the column does not invariably evoke a sensory response. This might be taken to suggest that the microvilli and the free

borders of the cell membrane are not involved in chemoreception, and it is therefore probable that the chemoreceptors involved in the pre-feeding response are the ciliated cells of the column.

A number of gland cells containing large electron-dense vesicles are also encountered in the column ectoderm of Tealia. These seem to correspond to the "homogene Drüsenzellen" described by the Hertwigs (1879), and probably secrete mucus onto the surface of the column. These gland cells are more obvious in Fig. 3.8 which again depicts the column ectoderm of Tealia. In addition, this electron-micrograph clearly shows the junctional complexes which exist between opposing membranes of the epithelial cells immediately below their free surfaces. These electron-dense complexes correspond to the "terminal bars" first demonstrated by classical cytological techniques, and are believed to represent specialized sites of firm attachment between cells. The resolution of the micrograph does not allow one to determine whether tight junctions (zonula occludens) are present in these complexes, but intermediate junctions (zonula adherens) and desmosomes (macula adherens) seem to be represented. The lateral membranes are considerably folded and this might provide a mechanism whereby rapid volume changes may occur in the epithelial cells. No structure that could be positively identified as a nerve cell was seen in the column ectoderm. Moreover, no connections were seen between the endodermal neural elements and the ectodermal ciliated cells. As the latter seem to form morphological connections with adjacent cells in the ectodermal epithelium, it is probable that they transduce sensory

information into electrical activity which is then propagated throughout the ectodermal epithelium. Since this is believed to be the site of the SSl this strengthens the conjecture that the ciliated cells of the column ectoderm are the chemoreceptors involved in the pre-feeding response.

DISCUSSION

One of the most interesting features that has emerged from these studies is that the column of Tealia has been shown to be highly receptive to a specific factor present in dissolved food substances. Much of the work on chemoreception in coelenterates has been directed towards the identification of compounds capable of eliciting feeding activity (see review by Lindstedt, 1971a). The chemoreceptors involved appear to be highly specific, responding exclusively to either a single compound, or at most a small group of closely related compounds. The feeding response is normally elicited by contact of the tentacles and oral disc with the feeding activators concerned, and recent work by Reimer (1971c) has shown that the feeding chemoreceptors in the zoanthid Palythoa psammophilia are in fact located on the oral disc and tentacles. These receptors appeared to be particularly concentrated on the mouth borders and peristome, and no obvious response could be elicited by contact of feeding activator with the column of the polyp. In virtually all the studies on feeding responses only behavioural observations have been employed as the measured output of the response, and it is perhaps unfortunate that none of these findings seem to have been followed up by

electrophysiological or morphological studies.

From the results obtained so far, however, it appears that coelenterates possess chemoreceptors that respond only to highly specific substances, and this seems to be particularly true of sea anemones. Ross & Sutton (1961a) have shown that detachment of the pedal disc and subsequent "shell-climbing" in Calliactis parasitica is dependent on the sensory recognition of a molluscan "shell factor", firstly by the tentacles and later by the pedal disc. This would imply that chemoreceptors capable of responding specifically to this "shell factor" must be located both in the pedal disc and the tentacles. The "shell factor" itself has not been identified, but it is known to be a stable organic substance found in shell and periostracum of both gastropods and lamellibranchs, and is insoluble in acetone, ether and alcohol. It is not digested by trypsin, however, which would suggest that it is not a simple protein. In C. polypus and C. tricolor the response to molluscan shells is still present but much weaker, especially in C. polypus. (Cutress & Ross, 1969; Cutress, Ross & Sutton, 1970; Ross, 1970). In these species the commensal crabs appear to play the primary role in establishing the association, rather than the anemone responding to an insoluble organic "shell factor". McFarlane (1969c) has shown that the "shell response" of C. parasitica involves excitation of the SS1, although it is not known whether shell contact or the tentacle adhesion process is responsible for evoking SS1 activity. It is clearly possible that specific chemoreceptors in the tentacles of C. parasitica are capable of directly exciting the SS1 when responding to "shell factor", in much the same

way as the pre-feeding chemoreceptors in the column of Tealia felina respond to dissolved food substances.

The swimming sea anemones Stomphia coccinea and Actinostola new species also show a "shell response", and as with Calliactis the chemoreceptors involved respond to different types of molluscan shell (Ross & Sutton, 1967a). The ability of the shells to elicit the response, however, seems to vary between shell types suggesting that the chemoreceptors are responding to a certain type of organic molecule which may have a slightly different configuration in different shells. The "shell response" in the actinostolids does not involve adhesion of the tentacles to the shell, unlike the response in Calliactis, and this would imply that the tentacles contact the shell surface for sensory purposes alone. In loosely attached specimens this behaviour often results in detachment of the pedal disc, and it would therefore be of great interest to establish whether SSl activity is evoked as a result of this sensory contact between tentacle and shell. The pedal disc of Stomphia, like that of Calliactis, also seems to possess chemoreceptors which recognize the "shell factor".

The swimming response of both Stomphia and Actinostola may be triggered by several substances contacting the tentacles of the anemone (Yentsch & Pierce, 1955; Sund, 1958; Robson, 1961b; Ward, 1965). Swimming is evoked by contact with the starfishes Dermasterias imbricata, Hippasteria spinosa and H. phrygiana. In addition, Actinostola can be induced to swim on contact with the submarginal surface of Stomphia (Ross & Sutton, 1967b), although Stomphia does not swim on contact with Actinostola. A swimming response may also be

evoked by contact of the column of Stomphia with the foot of the nudibranch Aeolidia papillosa (Robson, 1961c). In this case, however, swimming is not evoked when the nudibranch contacts the tentacles of Stomphia and this would suggest that the stimulatory substance produced by the nudibranch is different from that produced by the three starfishes. Furthermore, both substances would seem to act on different receptor sites. It is very interesting to note that, unlike the starfish extracts, the active extract from the nudibranch parallels the pre-feeding activator for Tealia in some ways. Both extracts are heat stable and both act on chemoreceptors located in the column. Chemoreception obviously plays a primary role in initiating the swimming response, but once again further research is hindered by the fact that the chemical activators involved have not been identified.

The "shell response" in Calliactis, Stomphia and Actinostola involves recognition of an unidentified "shell factor" by chemoreceptors located on the tentacles and pedal disc. In all three species contact of the tentacles with the surface of the molluscan shell eventually leads to detachment of the pedal disc. The "swimming response" in Stomphia and Actinostola involves recognition of unidentified substances, and this is also followed by pedal disc detachment. In Calliactis parasitica detachment of the pedal disc seems to be coordinated by activity in the SSl (McFarlane, 1969c), and it is perhaps not unreasonable to assume that pedal disc detachment in Stomphia and Actinostola may also be coordinated by the SSl. It would seem, therefore, that the chemoreceptors involved in all these cases are capable of feeding specific

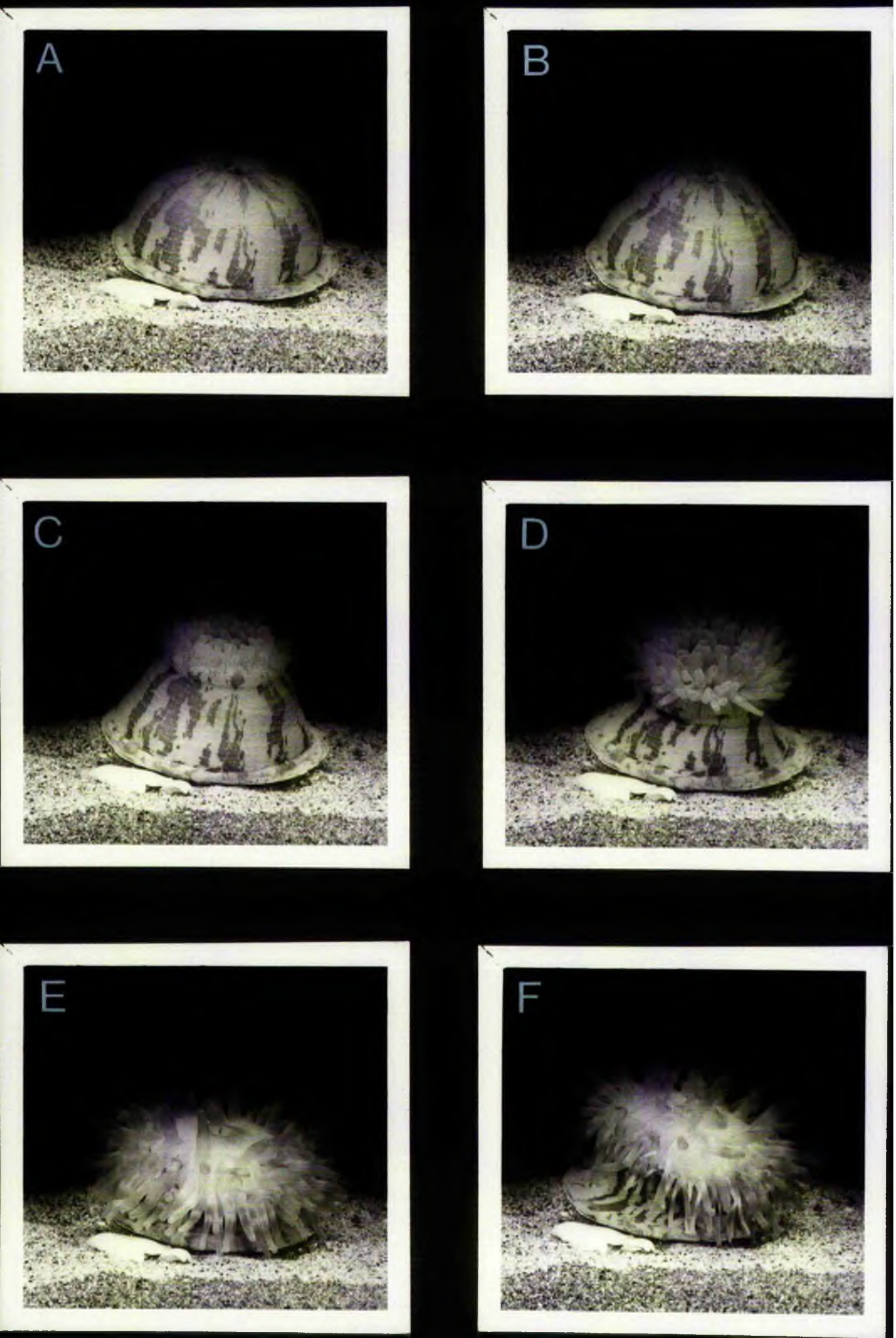
sensory information into the SSl, and in this respect they parallel the pre-feeding chemoreceptors located in the column of Tealia.

The stimulating chemicals have not been identified for any of these SSl chemoreceptors. This is in marked contrast to the feeding chemoreceptors where several chemical feeding activators have been identified in many different anthozoans and hydrozoans. The identification of the specific chemicals that activate the SSl chemoreceptors in sea anemones is obviously the next major obstacle to be overcome before we can begin to understand chemoreception and its role in the behaviour of these animals.

Although the results presented in this section and in Part Two indicate that the SSl plays an important role in preparatory feeding activity, it becomes increasingly obvious from behavioural observations that certain responses cannot be explained on the basis of SSl activity alone. Careful observation of intact animals engaged in pre-feeding activity reveals that endodermal muscular elements must be involved in certain sequences. Fig. 3.9 depicts a sequence in which a closed Tealia opens and then expands its oral disc in response to a piece of Mytilus tissue placed near the base of the column. McFarlane & Lawn (1972) have pointed out that expansion of an open anemone need involve only the ectodermal radial muscles situated in the oral disc, whereas the opening of a closed anemone would seem to involve relaxation of the sphincter and retractor muscles, both of which are endodermal in origin. As yet there is no unequivocal evidence for an endodermal inhibitory action by the SSl (McFarlane, 1973a) and it is obvious that future work on pre-feeding activity

Fig. 3.9. Closed Tealia opening in response to food placed near the base of the column. The anemone had been closed for at least 2 hr before the experiment was commenced. (A) The closed anemone immediately after the food had been placed close to the base of the column. (B) The sphincter begins to relax and the tips of the tentacles begin to emerge. (C) The sphincter and retractors continue to relax and the collar begins to twist down towards the base of the column. The oral crown continues to emerge. (D) The collar is now pointing downwards and the tentacles are almost fully exposed to view. (E) Expansion of the oral crown is complete. The tentacles and oral disc are well expanded in order to increase the range for food capture. (F) Later stage of the pre-feeding response in which the pharynx begins to protrude above the level of the oral disc. Time from A to F = 3 min.

3.9

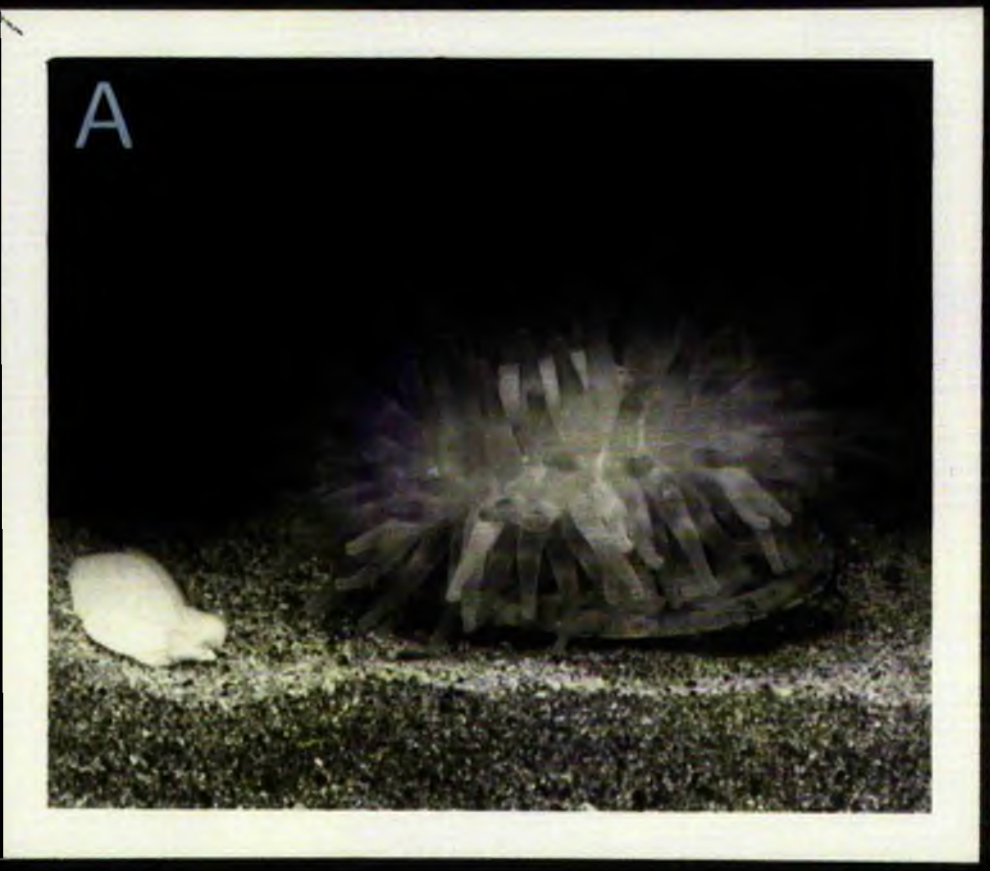


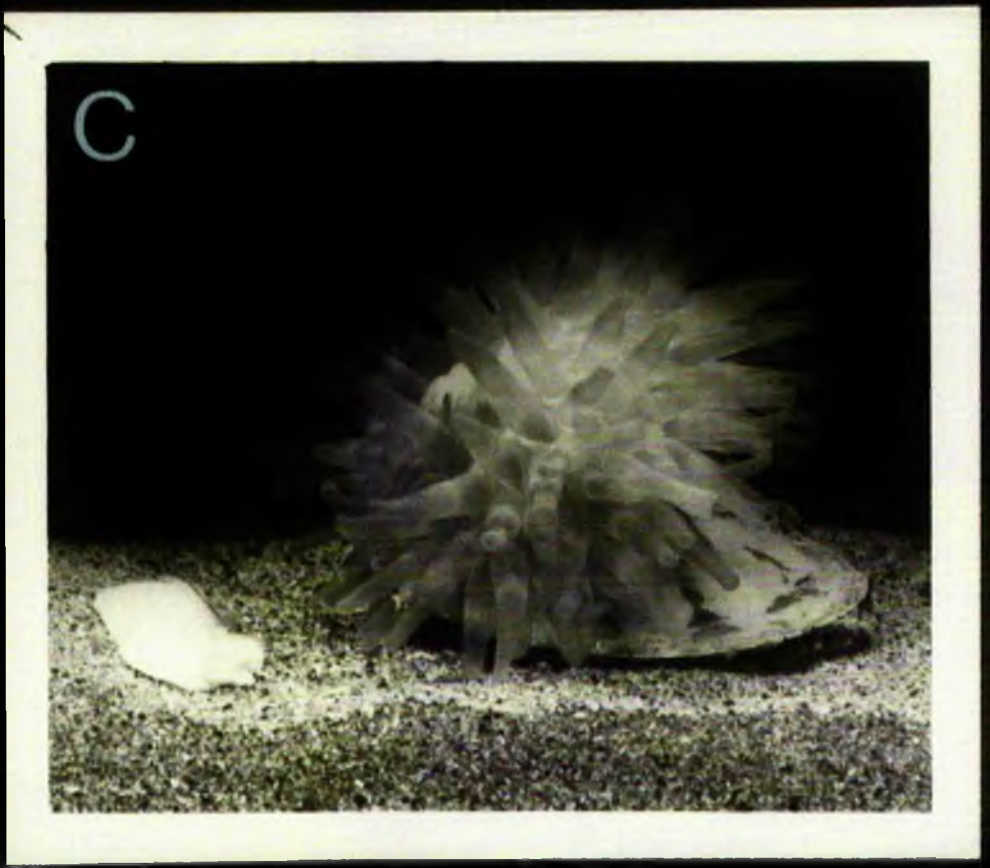
should be directed towards explaining the means by which the sphincter and retractors may relax when the column is exposed to food substances. In this respect it is interesting to note that in Hydra the body contractions induced by mechanical agitation or by light are inhibited in the presence of reduced glutathione (Rushforth, 1955).

Relaxation of the sphincter and retractors in closed anemones during the pre-feeding response is not the only problem that awaits further analysis. Excitation of endodermal musculature may also occur when the animal extends its column and starts to "sway" in response to food substances, thereby increasing its chances of contacting solid food nearby. Fig. 3.10 shows such a sequence in which the anemone bends to one side in an unsuccessful attempt to contact food lying near the base of the column. These "swaying" movements, however, do not invariably accompany expansion of the oral disc during the pre-feeding response. In this particular sequence the anemone was induced to bend towards the food by a very light touch to the column with a clean glass rod. Local contractions of the parietals and parieto-basilars then seem to be gradually propagated along that side of the column receiving the mechanical stimulus, and this results in unilateral bending of the column. In the absence of dissolved food substances a similar light touch to the column produces no observable response, or at most a slight local contraction immediately beneath the point of stimulation. In this case, the contraction does not spread along the column, and hence unilateral bending does not occur. These observations would indicate that exposure to food substances makes the column very sensitive to mild mechanical stimulation,

Fig. 3.10. Column extension and bending during the pre-feeding response. (A) Expanded anemone before bending begins. (B) Oral crown begins to dip towards the food source owing to a small unilateral contraction of the column. (C) Column extension and bending becomes more distinctive. (D) Extreme stage of column extension and bending, bringing plane of oral disc almost perpendicular to substrate. Note protrusion of pharynx at this stage. Time from A to D = 2 min.

3.10





and this is probably closely linked with the "swaying" movements often observed during pre-feeding activity.

Extension of the column is also a result of excitation of endodermal musculature. This is presumably accomplished by a slow tonic contraction of the column circulars, but once again the pathway that mediates this response is unknown. Much work still remains to be done, therefore, before a complete understanding of the pre-feeding response can be obtained. A similar situation applies to the feeding response, which involves the capture and engulfment of solid food. It is to be hoped that from a detailed analysis of such behaviour, it will eventually prove possible to explain complete sequences of behavioural activity in coelenterates in terms of known receptors, conduction systems and effectors.

SUMMARY OF PART THREE

1. Single, localized applications of food extract to the column of Tealia felina often have long-lasting effects in which the sensory response may continue for periods in excess of 1 hr.

2. The sensory response seems to show genuine sensory adaptation, for the decrease in frequency of evoked pulses is not related to fatigue in the conduction system involved. This sensory adaptation is unusual in that it operates on a much extended time-scale compared with the same phenomenon encountered in higher animals.

3. All the evoked SPIs appear to originate from the site of the applied chemical stimulus. This indicates that the activity is not due to indirect mechanical stimulation and implies that a purely chemosensory response is involved.

4. Evidence from electrophysiological studies indicates that the chemoreceptors involved in the pre-feeding response are dispersed throughout the column of Tealia, and are absent from the pedal disc and pharynx.

5. The pre-feeding activator present in the food extract is an unidentified compound with a molecular weight of less than 1000. It is heat stable and soluble in water and acetone.

6. Ultrastructural evidence is presented to support the conjecture that the pre-feeding chemoreceptors are ciliated cells located in the ectodermal epithelium of the column.

GENERAL DISCUSSION

The results presented in this thesis have already been discussed at some length in each of the preceding sections. This discussion, therefore, will attempt to deal with more general aspects of behavioural physiology in the anthozoans, with special regard to the evolution of complex behaviour. Before complex behaviour patterns can emerge, however, the physiological machinery available must also evolve in such a way that it is capable of responding differently to different types of stimulation. The study presented in Part Two has shown that the ectodermal radial muscles of the oral disc in Tealia felina are representative of a coelenterate effector system which possesses the ability to respond to different modes of input in several ways. The multiple responses shown by the oral disc radials in Tealia may be summarized as follows:

1) Local activity; myoid or mechanical conduction?

2) Spontaneous activity; myogenic or local nerve-net?

3) Fast contractions; symmetrical and coordinated by the through-conducting nerve-net.

4) Slow contractions; symmetrical and coordinated by the through-conducting nerve-net.

5) Induced relaxation; probably symmetrical and coordinated by the SSI.

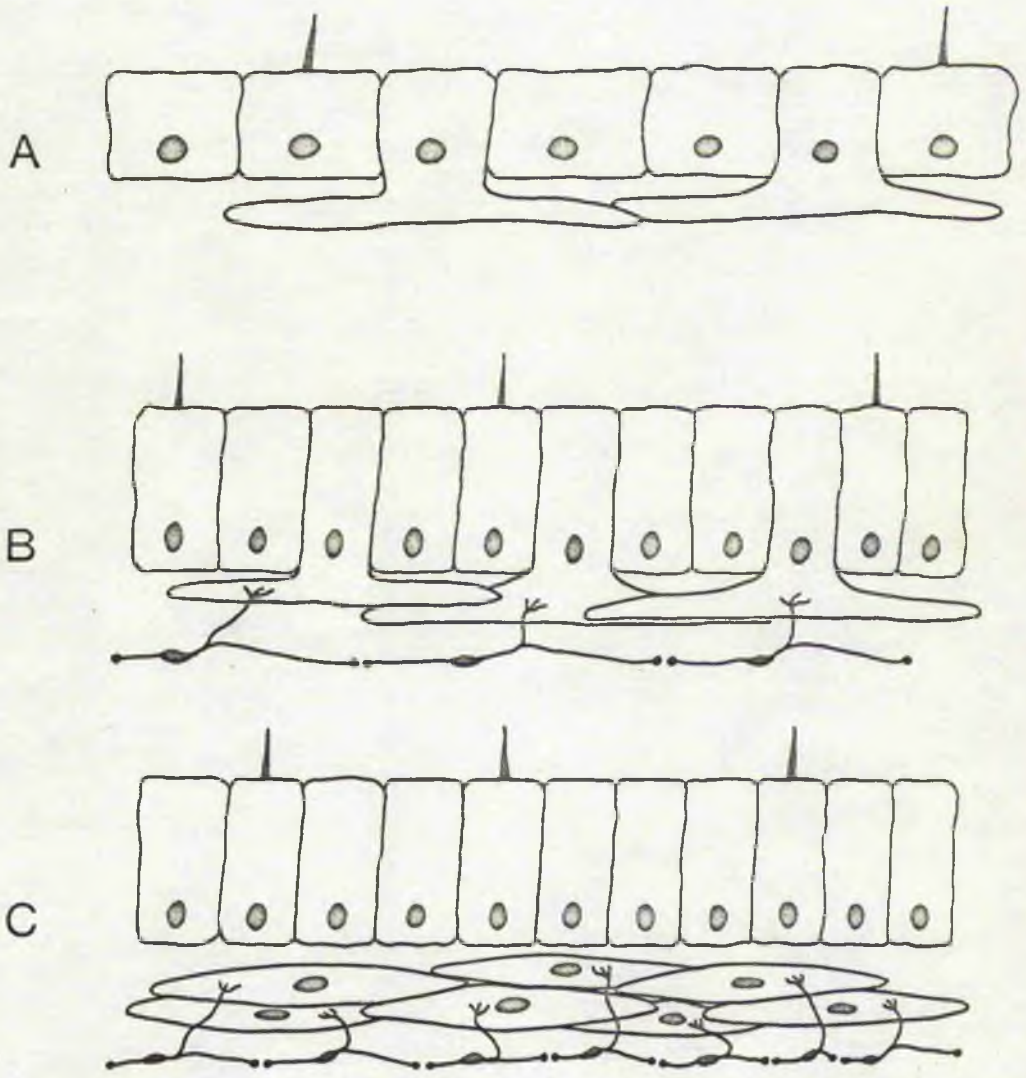
The scheme depicted in Fig. D.1. is offered as just one explanation of how such an advanced effector system may have evolved from the hypothetical primitive state, and should be regarded as pure speculation in the absence of further information. Kleinenberg (1872) first appreciated

that the epithelio-muscle cell represents a primitive combination of receptor, conductor and effector, and Horridge (1968a) has pointed out that such cells are likely to constitute a fundamental unit in the evolution of conduction-effector systems. Pantin (1956) has suggested, however, that the action of a single epithelio-muscle cell would be negligible unless it were connected to other cells of the same type, thereby forming a primitive muscle field. The real effector, therefore, would be the entire muscle field, and it is conceivable that in addition to mechanical coupling, some form of electrical coupling may develop between the constituent cells in order to propagate and coordinate the activity of the contractile elements.

In recent years it has been shown that simple epithelial cells are capable of conducting electrical events in an unpolarized non-decremental fashion, as in the epithelium covering the swimming bells of siphonophores (Mackie, 1965). In the hydromedusan Sarsia (Mackie & Passano, 1968) it has been shown that the simple epithelium of the exumbrellar ectoderm is capable of conducting all-or-none impulses to the radial muscles in the subumbrellar ectoderm. This, therefore, could represent a primitive condition in which simple epithelial cells conduct excitation to regions of myoepithelium. Such a situation is depicted in Fig. D.1A. This hypothetical primitive stage would be capable of showing spontaneous activity, inherent in the individual epithelio-muscle cells; graded local contractions, transmitted as a result of the linking of muscle tails to form a primitive muscle field, and symmetrical contractions, probably with a protective function, coordinated by through-conducted impulses in the epithelial

Fig. D.1. Possible stages involved in the evolution of the ectodermal musculature of sea anemones. The scheme depicted here offers one explanation of how multiple responses may have developed in this effector system. (A) Primitive stage: simple epithelial cells conduct excitation to regions of myoepithelium; e.g. hydromedusan ectoderm. (B) Intermediate stage: the epithelial cells have become long and thin to allow for tighter packing of the muscle tails. Processes from the endodermal nerve-net have formed connections with the muscle tails in order to coordinate the muscles involved in the protective withdrawal response. This stage superficially resembles the system found in the ectoderm of Hydra. (C) Advanced stage: The muscle tails have separated from the epithelium, thereby increasing the efficiency of the ectodermal musculature. The nerve-net continues to innervate the musculature and mediates both fast and slow symmetrical contractions. The ectodermal epithelial cells retain their capacity to conduct electrical activity in a non-decremental fashion and hence constitute the seat of the SSl. This stage represents the arrangement found in the ectoderm of the oral disc and tentacles of most sea anemones.

D.1



cells. Such a stage may be represented by the ectoderm of the hydromedusan Sarsia. It is also possible that some of the epithelial cells would function as receptors of some sort, feeding sensory information into the epithelial conduction system.

Let us now suppose that the next evolutionary trend was towards enhancing the potency of the muscular system by increasing the density of contractile elements in the muscle fields. Remembering that all structures in the coelenterate body plan are built from cell-layers that must remain a single cell thick, the most efficient method of increasing the cross-sectional area of the musculature is for the underlying mesogloea to be thrown into a series of folds. In this way the muscle elements attached to the mesogloea would effectively increase their cross-sectional density to produce a more powerful muscle field. Such a system seems to have evolved in the endodermal musculature of sea anemones, where surprisingly powerful muscles, such as the mesenteric retractors, have been developed in this manner. The folding of the cell-layers in this way is well suited to the functions of the endodermal epithelium, where the increased surface area enhances such processes as digestion and absorption.

A different problem is faced by the ectodermal epithelium, however, for it is presumably desirable that a flat surface-layer be maintained for the efficient functioning of such structures as nematocysts, spirocysts and sensory cells. If the ectodermal epithelium were to become greatly folded during contraction, in a similar

manner to the endodermal epithelium, it is possible that the nematocysts and spirocysts might distort and discharge their contents prematurely. It is also conceivable that the delicate sensory cells might become damaged. For these reasons the ectodermal epithelial cells may have adopted a different approach to the problem of increasing the potency of the muscle field. It is possible that this was achieved by a tendency for the muscle tails to become tightly packed together, thereby increasing the density of muscle elements in the myo-epithelium. From a consideration of the three-dimensional geometry of the epithelio-muscle cell, the major factor limiting the degree of aggregation between adjacent muscle tails would be the width of the surface area of the epithelial portion of the cell. It is postulated, therefore, that there was a tendency for this part of the cell to become longer and narrower to facilitate the packing together of the muscle tails. This may explain why the cells encountered in the ectodermal epithelium of sea anemones tend to be long and thin.

This method of increasing the efficiency of the ectodermal musculature would obviously be less effective than that adopted by the endodermal epithelium, where a much greater density of contractile elements may be achieved merely by increasing the degree of folding. One can thus envisage a situation in which the endodermal musculature becomes more and more effective in relation to the ectodermal musculature. If one assumes that in the primitive condition longitudinal contractions were mediated by the ectodermal musculature, and antagonistic circular contractions were mediated by the endodermal musculature, then it may be

postulated that as the endodermal myoepithelium increased its powers of contraction there was an accompanying tendency for the muscle fields to separate and adopt different gradients of growth. In this way, one could envisage the emergence of the first signs of differentiation in the endodermal musculature, and this would eventually lead to the development of highly effective endodermal longitudinal muscles. Such muscles would be the precursors of the mesenteric retractors and the parietals, and it is interesting to note that these seem to be directly associated with the mesenteries. The retractor is inserted on the endocoelic face of the mesentery itself, and the parietals lie along the line of insertion of the mesentery into the column wall (Stephenson, 1928).

The development of powerful longitudinal muscles in the endoderm would presumably render the inefficient ectodermal myoepithelium redundant, and one might expect that eventually the ectodermal muscle tails would degenerate and finally disappear altogether. This would apply to the ectoderm of the pedal disc and column, where the endodermal longitudinals could easily assume the same role at that previously adopted by the muscle elements of the ectoderm. In the tentacles, however, there are no mesenteries along which the endodermal longitudinals may become attached. This may explain why the tentacle longitudinal muscles have remained ectodermal. The oral disc radial muscles may also be regarded as part of the same muscle field (McFarlane & Lawn, 1972). We therefore have a situation in which the endodermal myoepithelium is responsible for the development of both circular and longitudinal muscles in all regions of the anemone apart from the tentacles and oral disc, where the

longitudinal musculature is ectodermal. This type of muscle arrangement is encountered in the majority of sea anemones except those in the family Gonactiniidae, where longitudinal muscle fibres are found in the ectoderm of the column (Robson, 1971).

Accompanying the development of the musculature in the endoderm one might expect the first appearance of recognisible nerve cells. The possible methods by which nervous systems may have evolved in coelenterates have been ably discussed by Parker (1919), Pantin (1956) and Horridge (1968a), and will not be reiterated here. One can assume that the nerve-net developed as a conduction system capable of transmitting excitation along restricted pathways from one region to another at much higher velocities than those encountered in epithelial conduction systems. Eventually, a through-conduction system may have developed in this nerve-net to coordinate the muscles involved in the protective withdrawal response. This would mean that the endodermal nerve-net would have to form a connection with the ectodermal longitudinal muscles in the oral disc and tentacles. This stage is depicted in Fig. D.1B and superficially resembles the system found in the ectoderm of Hydra.

Once the ectodermal musculature is connected with the through-conduction system, either fast or slow contractions may be mediated by the nerve-net by utilizing a system of temporal integration. The ectodermal epithelial conduction system, therefore, would no longer serve a useful function in coordinating symmetrical slow contractions of the ectodermal myoepithelium. Hence, the muscle tails would now be able to physically isolate themselves from the epithelial

portion of the cells in order to create a more efficient muscle field, composed of densely-packed longitudinal fibres. This muscle would be able to contract quite strongly without seriously distorting the ectodermal epithelium situated immediately above. One finds such an arrangement in the ectoderm of the oral disc and tentacles of most sea anemones, and this "advanced" stage is depicted in Fig. D.1C.

It is suggested that the isolation of the muscle tails from the overlying epithelium represents a significance stage in the evolution of an inhibitory system mediated by the SS1. If one concedes that the SS1 constitues an ectodermal epithelial conduction system, it is conceivable that the muscle elements and the SS1 would no longer remain electrically coupled once the muscle tails have become physically isolated. This would imply that excitation in the SS1 could no longer directly evoke contraction of the ectodermal musculature, but instead may effectively remove calcium ions from the vicinity of the muscles, in the manner outlined in Part Two, and thereby induce a state of inhibition. It must be emphasised, however, that to date there is little or no direct evidence to support or refute these ideas.

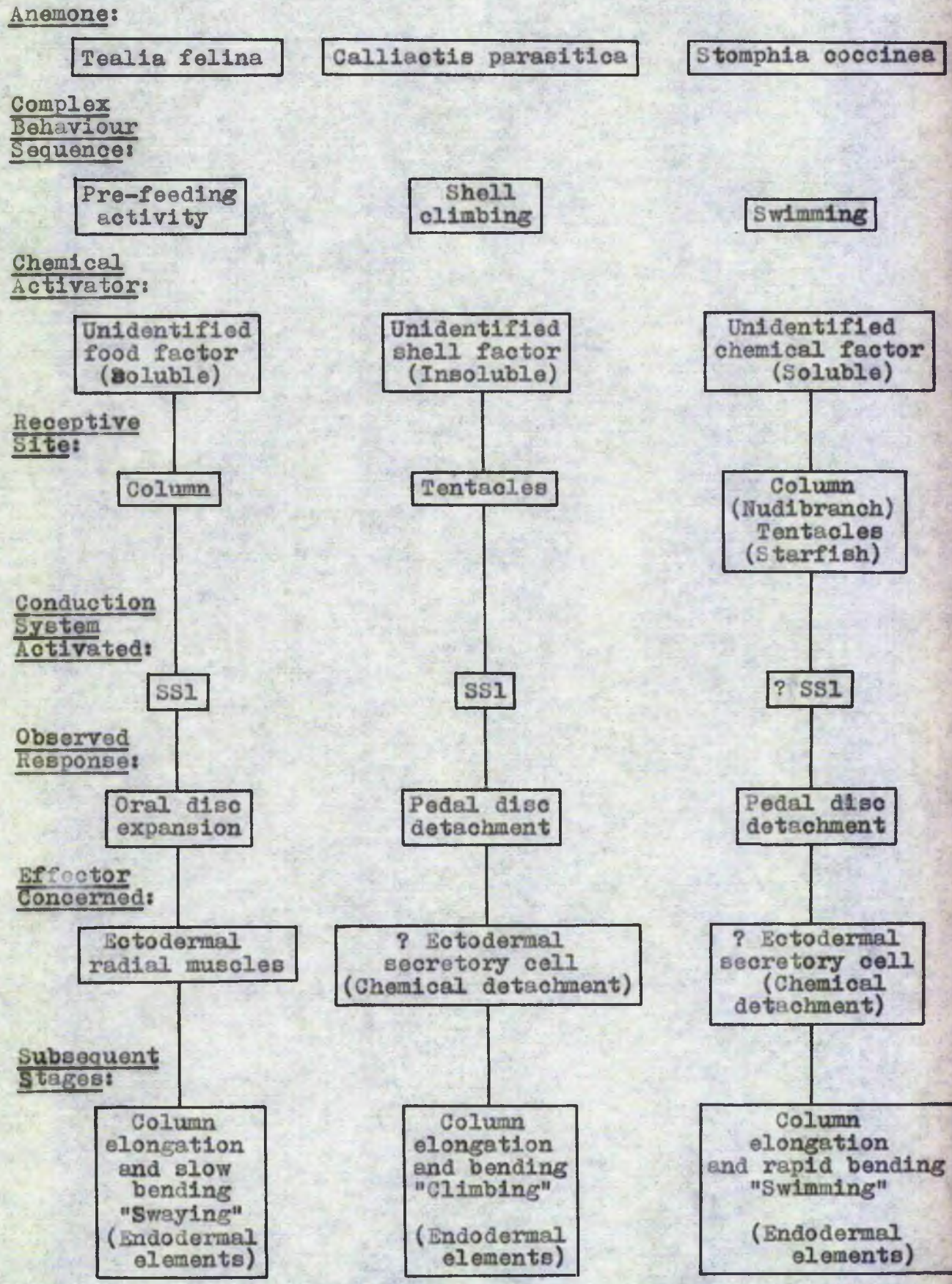
As far as the inhibitory response in the sphincter of Calliactis is concerned (see Part One) I think that one must assume that this appeared as the nerve-net developed into a more sophisticated conducting pathway, capable of controlling and coordinating increasingly complex behaviour patterns. The fact that the sphincter is enclosed in tubes within the mesogloea, and therefore presumably isolated from the influence of epithelial conduction systems, may explain

why it was necessary for the inhibitory response to be mediated by nerve fibres innervating the sphincter.

The development of more intricate coordinating pathways was probably accompanied by a tendency for the sensory elements in the ectodermal epithelium to become more specialized. It is probable that some receptor cells continued to feed sensory information directly into the epithelial conduction system, whereas others may have linked up with the underlying neural elements, thus enabling sensory information to be conveyed from ectoderm to endoderm. Leghissa (1965) describes connections between processes from ectodermal receptor cells and elements of the ectodermal nerve-net in the tentacles of Actinia equina. It has been shown that in Mimetridium cryptum the ectodermal nerve-net of the oral disc and tentacles is directly linked with the retractor nerve-net of the mesenteries (Batham, 1965). This wouldmpresumably provide a pathway by which sensory information from ectodermal receptors could be transmitted to endodermal elements and thereby influence the activity of endodermal musculature. Robson (1965) has suggested that similar pathways between ectodermal and endodermal elements may exist in the pedal disc of Calliactis, where muscle fibres from the retractors and parietobasilars penetrate the mesogloea and extend "almost if not quite" to the pedal ectoderm. A similar situation is also encountered in the pedal disc of Stomphia (Robson, 1961b).

It was emphasised in Part Three that several complex behaviour sequences in sea anemones were initiated by the recognition of specific substances impinging on chemoreceptors located in the ectoderm. The subsequent stages

Fig. D.2. Summarized analysis of some complex behaviour sequences in sea anemones.



in the behavioural sequence always seem to involve excitation of the SS1, accompanied by some appropriate response in an ectodermal effector, and activation of endodermal musculature, involving extension and bending of the column. It is probable, therefore, that during these complex sequences ectodermal receptor cells are able to feed sensory information into conduction systems located both in the ectoderm and the endoderm. The sensory elements responsible for monitoring changing events in the external environment are obviously vital to the functioning of those systems responsible for coordinating sequences of complex behaviour.

Fig. D2. gives a summarized analysis of complex behaviour sequences encountered in Tealia felina, Calliactis parasitica, and Stomphia coccinea. One cannot fail to notice some astounding similarities between pre-feeding activity, "shell-climbing" and "swimming", all of which, to the casual observer, would appear to be completely different behaviour patterns. One question that seems to arise from such a comparative analysis is this: if the SS1 coordinates pedal disc detachment in Calliactis parasitica (and also perhaps Stomphia coccinea) why does Calliactis not become detached during pre-feeding activity?

Several workers in the past have described typical pre-feeding responses in a variety of species, such as the "common" sea-anemone (Pollock, 1883), Metridium (Allabach, 1905) and Actinia (Piéron, 1906). It would appear that pre-feeding activity is a basic response found in most sea anemones, and Calliactis parasitica is no exception, for it responds to dissolved food substances with SS1 activity and associated expansion of the oral disc (McFarlane, personal

communication). In C. parasitica the frequency of SS1 activity evoked during the pre-feeding response rarely exceeds 1 SP1 every 30 sec, whereas McFarlane (1969c) has shown that detachment in this species only occurs at frequencies in the range of 1 SP1 every 3 sec and 1 every 10 sec. It is probable that different ectodermal receptors are involved in each case, and this might explain the observed differences in firing frequency. The fact that the "shell response" involves contact chemoreceptors located in the tentacles, and the pre-feeding response involves distance chemoreceptors located in the column reinforces the idea that different sensory elements may be involved. It has been observed on occasions that certain specimens of Calliactis sometimes detach while being fed, and this underlines the observation that as far as the SS1 is concerned the only difference between the two responses is one of impulse frequency.

One can do little more than speculate on the ways in which these complex behaviour patterns might have evolved. Starting from the assumption that pre-feeding activity is a basic response found in all sea anemones, one must search for signs of the first appearance of pedal disc detachment. If one closely observes anemones engaged in feeding activity one often notes that feeding is accompanied by a shedding of the mucus coat surrounding the column. Occasional reference to this phenomenon can be found in some of the earlier literature. Sir John Dalyell (1848) gives a general account of the natural history of sea anemones in which he remarks that "the skin is cast very often, especially after feeding greedily". It is conceivable that the shedding of the mucus

coat is somehow related to the SSl activity elicited during the pre-feeding response. This phenomenon could represent the precursor of pedal disc detachment, which may also involve a similar process; in this case the cementing layer attaching the pedal disc to the substrate is shed during detachment.

Many sea anemones are capable of detaching from the substrate and moving to new positions by slowly creeping or gliding about on the pedal disc. In selecting a new substrate on which to settle some sea anemones show a marked preference for molluscan shells. For example, Metridium senile shows a distinct preference for molluscan shells (McFarlane, personal communication), and both Stomphia and Actinostola exhibit a very purposeful shell response (Ross & Sutton, 1967a). It seems likely, therefore, that detachment of the pedal disc and preferential settling on molluscan shells may be primitive response common to many species of sea anemones. McFarlane (1969c) has suggested that C. parasitica may have modified this response in order to overcome the difficulties encountered in settling on the moving shell of a hermit crab. Such modifications would include initial adhesion of the tentacles to the shell surface and the development of a complex series of columnar bending movements that would eventually bring the pedal disc into contact with the shell. One would expect that the development of a rapid detachment process in Calliactis would be accompanied by the development of highly specialized chemoreceptors in the tentacles, capable of eliciting high-frequency SSl activity in response to the shell factor. In this way, the ectodermal receptor-conduction

system may have developed the capacity to coordinate a number of responses in different effectors merely by altering the frequency of conducted impulses. This mode of control, of course, is also employed by the nerve-net and underlines the way in which sea anemones have fully utilized the possibilities of temporal integration. Presumably, this system has developed because of the nature of the anthozoan body plan which offers little or no opportunity for spatial integration of conducted activity.

The most rapid detachment process of all, that in which Stomphia and Actinostola become detached from the substrate during the swimming response, probably represents the culmination of this line of development. It has not yet been established whether or not a slow conduction system exists in Stomphia, although Ross & Sutton (1964a) provide evidence that a conduction system separate from that which evokes retraction controls the swimming response. A few suitably applied electrical shocks (6 to 8 at a frequency of 1 every sec) are the only stimuli required to elicit the complete response, which involves rapid detachment and vigorous swimming movements. Such a system constitutes a superbly adapted protective response, for it would appear that the complete behavioural sequence may be triggered by a few high-frequency SPIs (assuming, of course, that the SSl is involved in this case). Presumably, some form of direct or indirect input must also permeate the endodermal elements in order to coordinate the activities of the parieto-basilar muscles during the swimming flexions.

In contrast, the "shell-climbing" response of Calliactis cannot be elicited by a simple series of

electrical stimuli. This reinforces the idea that "shell-climbing" is not merely a series of simple reflexes, but requires a continual flow of sensory information before the response can proceed to completion. This information is presumably supplied by receptors in the tentacles contacting the shell. Ross & Sutton (1961a) have shown that if the contact between tentacles and shell is broken the whole programme of shell-climbing activity is abandoned.

Much useful information can be obtained from a comparative analysis of complex behaviour patterns in sea anemones. One of the most crucial problems awaiting further analysis will be to determine whether or not SS1 activity is involved in the swimming response of Stomphia. Another complex behaviour sequence worth examining with electrophysiological techniques is the "aggressive response" encountered in some sea anemones possessing acrorhagi (Abel, 1954; Bonnin, 1965; Francis, 1973a,b). In this rather dramatic response the "victim" of the "aggressive" activity often detaches its pedal disc from the substrate and gradually moves away from the "aggressor". It would be of great interest to establish whether the application of acrorhagi to the column of one of these anemones elicits activity in the SS1, thereby inducing detachment of the pedal disc. It might also prove useful to apply recently developed recording techniques to the study of coordinated responses in colonial anthozoans. The conduction systems that coordinate activity between individual polyps in coral colonies should obviously be examined, since indirect observations (Horridge, 1957) have revealed that the spread of excitation in different species of coral shows considerable variation.

As Horridge (1968b) has pointed out: "The recent discoveries of cell-to-cell transmission in hydroid polyps and of potentials that appear to be propagated in sheets of non-nervous cells ..... are bound to influence further work on anemones and corals, in which such possibilities have hardly been considered". It is to be hoped that the work presented in this thesis has made some contribution towards our understanding of those processes which control and coordinate behavioural activity in sea anemones. With the development of increasingly sophisticated behavioural and electrophysiological techniques the immediate outlook for research in this field seems very promising indeed. When one reflects on the behavioural complexity exhibited by these fascinating creatures, however, one is led to the inevitable conclusion that the ultimate goal of behavioural physiology - that of explaining the complete behaviour of an animal in terms of known excitable elements - may seem tantalizingly close, but will continue to remain unattainable for quite some time.

GENERAL SUMMARY

This study has employed a variety of techniques in an attempt to further our understanding of the mechanisms which control and coordinate behavioural activity in sea anemones. It has proven possible to examine the properties of receptors, conductionnsystems and effectors in these animals, and the information acquired from such investigations has provided the basis upon which certain aspects of behavioural activity have been explained. Some of the more important results and conclusions derived from this study may be summarized as follows:

1) An inhibitory response has been demonstrated for the first time in the marginal sphincter of Calliactis parasitica. This response is capable of delaying the initiation of slow contractions for periods of upto 30 min or more. Fast contractions of the sphincter, however, seem to remain completely unaffected during the period of inhibition.

2) The inhibitory effect is mediated by the through-conducting nerve-net and indirect observations indicate that the process may involve release of inhibitory transmitter at the neuromuscular junction.

3) From observations of spontaneous electrical activity in unstimulated preparations it has been shown that inhibitory responses may play an important role in the control and coordination of basic behavioural activity, such as slow closure sequences.

4) The nature of the mechanisms involved in the

control and coordination of oral disc activity in Tealia felina has been investigated. It has been demonstrated that the ectodermal radial muscles of the oral disc show several different types of activity. These consist of spontaneous and local activity, fast and slow symmetrical contractions, and induced relaxation.

5) The radials of the oral disc appear to have a dual control system, excitatory from the nerve-net and inhibitory from a slow conduction system - the SS1. Should the SS1 prove to be a neuroid conduction system then this would be the first demonstration of muscular inhibition by such a system.

6) The SS1 coordinates relaxation and inhibition of spontaneous activity in the oral disc radials. The frequency range of SS1 activity capable of eliciting these inhibitory responses extends from 1 pulse every 2 sec to 1 pulse every 60 sec.

7) Application of dissolved food substances to the column of Tealia felina evokes activity in the SS1. This chemosensory response shows a genuine sensory adaptation that operates over a much extended time-scale compared with sensory responses of higher animals. The response may continue for periods in excess of 1 hr.

8) Electrophysiological studies indicate that the chemoreceptors involved in this response are dispersed throughout the column ectoderm of Tealia, and are absent from the pedal disc and pharynx.

9) Studies on the ultrastructure of the column region indicate that the chemoreceptors may be ciliated

cells situated in the ectodermal epithelium.

10) These observations are discussed in relation to the pre-feeding response of Tealia. A model for oral disc expansion has been described and the mechanisms which control and coordinate activity in the oral disc have been re-examined.

11) The means by which complex behaviour patterns and mechanisms of control and coordination may have evolved in sea anemones are discussed in the light of this new information.

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