MULTIPLE PACEMAKERS AND THE BEHAVIOUR
OF THE HYDROID *TUBULARIA*

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INTRODUCTION

This paper deals with the behavioural physiology of the hydroid *Tubularia*. *Tubularia* is a favourable animal for behavioural analysis. It is a large hydroid and because it is sedentary, growing from a fixed stalk, it can be pinned down by the stalk in a dish of sea water and yet not be unnaturally restrained. It is readily available at many marine stations and, if necessary, it can be cultured indefinitely in the laboratory (Mackie, 1965*b*). The behavioural repertoire of *Tubularia* consists of only a limited number of activity patterns, although the patterns themselves may be fairly complex. And it is rather easy to record electrical activity from a *Tubularia* polyp which appears to be causally related to some aspects of its behaviour. Electrical correlates to some other behavioural events, however, have not yet been recorded.

There are a number of pacemakers in *Tubularia*. The term 'pacemaker', as used here, refers to those elements which spontaneously and repeatedly (although not necessarily rhythmically) initiate measurable events such as electrical potentials or muscle contractions. The term 'pacemaker system' will be used to refer to a pacemaker or group of pacemakers apparently serving a common function, and the conducting system involved in the propagation of electrical potentials initiated by this pacemaker activity. A previous paper (Josephson, 1962) describes electrical activity from one pacemaker system in *Tubularia*. This pacemaker system produces electrical potentials which are most easily recorded in the distal stalk, and which typically appear in a pattern of repeating single pulses interrupted at intervals by short bursts of three or more pulses. Three non-polarized conducting systems have been identified in the stalk of *Tubularia* (Josephson, 1965). One of these, the distal opener system (DOS), conducts at about 15 cm./sec. (19° C.). The DOS is found throughout the stalk and hydranth. Activation of the DOS causes synchronous depression of the distal tentacles of the hydranth. The second conducting system, the triggering system (TS), conducts at about 17 cm./sec. Activation of the TS can trigger electrical potentials in the neck of a polyp. The threshold of the TS to electrical stimuli is slightly lower than that of the DOS. The third conducting system, the slow system (SS), conducts at about 6 cm./sec. The SS threshold is about twice that of the DOS in a fresh preparation and it increases markedly with SS activation. The SS is quite labile, and often fires

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repetitively to supra-threshold stimuli. SS activation has no known behavioural correlate, nor does it appear to affect spontaneous electrical activity in the hydranth. The present paper gives an analysis of polyp behaviour, describes additional pacemaker systems found in the distal hydranth and tentacles, and describes some of the inter-actions which occur between pacemaker systems of a single polyp and between pacemaker systems of different polyps on a colony. Most of the activities to be described could be termed 'visceral', for they appear to be involved with food digestion and the transfer of the products of this digestion throughout the polyp.

MATERIALS AND METHODS

The hydroids principally used were *Tubularia larynx* Ellis & Solander,* collected from the northern end of the Cape Cod Canal. These animals were held in running sea water at Woods Hole, and were kept in still sea water at 19–20° C. when under observation. Some additional observations were made on *T. crocea* Aggassiz,* collected from the Port de la Darse at Villefranche-sur-Mer, and cultured for 10 weeks in the Station Zoologique of the Université de Paris at Villefranche.

The electrical recording techniques have been described previously (Josephson, 1965). Briefly, metal electrodes of either stainless steel or platinum-iridium were used, as well as plastic suction electrodes. The metal electrodes were electrolytically etched to tip diameters of 2–5 μ and insulated with lacquer. They were either rigidly mounted in a manipulator or flexibly mounted from a coil of fine copper wire. The suction electrodes were made by heating and drawing plastic tubing (Tygon) until it ended in a thin, hollow filament. The tip of the filament was cut off where it had an internal diameter of 30–90 μ. The drawn portion of the tubing was filled with sea water and a syringe was attached to the undrawn end. Electrical contact with the sea water in the tubing was made with a silver wire cemented through the wall of the tube. The tip of the electrode was placed against a part of the polyp and held in place by suction created by withdrawing the plunger of the syringe. In some cases the suction was calibrated by measuring the air volume of the system and the change in this volume produced by withdrawing the syringe plunger. Electrical potentials were amplified with capacitor-coupled amplifiers having long time-constants, and the final display was on an oscilloscope or pen-writer. Stalk stimuli were electrical pulses, 1 msec. in duration, delivered through a pair of silver wires insulated to the tip and placed one on each side of the stalk.

Suction electrodes of the type used are light, flexible, and self-adhering. They can be used to record from the surface of almost any part of a *Tubularia* polyp, including individual tentacles. Some caution, however, must be used in interpreting potentials recorded with a suction electrode on a *Tubularia* polyp. Brief, negative potentials begin to appear if the electrode suction is increased beyond some critical level. These potentials are quite local; they are seen from an electrode with excessive suction and not from an adjacent electrode (Fig. 5). The frequency of these potentials increases with increasing suction. The origin of the 'suction potentials' is not known; they are not seen from suction electrodes on the covered neck or stalk of *Tubularia* where the

* We wish to thank Dr W. J. Rees of the British Museum for identifying these species for us from preserved material.

electrode tip rests on a perisarc layer and not directly on the epithelium. Since these potentials appear to be a direct result of the suction and not to represent normal events in the animal, the electrode suction in the present study was kept low, generally between 40 and 50 mm. Hg, in order to avoid them.

<div align="center">RESULTS</div>

<div align="center">I. *Behaviour of* Tubularia *polyps*</div>

The principal structures of a *Tubularia* polyp are shown in Fig. 1. The gonophores, which are borne just distal to the proximal tentacles, have been omitted from the drawing. The stalk (hydrocaulus) arises from a stolen (hydrorhiza). The stalk first constricts slightly and then enlarges again before joining the hydranth. The distal portion of the stalk, beginning at the proximal edge of the constriction, will be termed
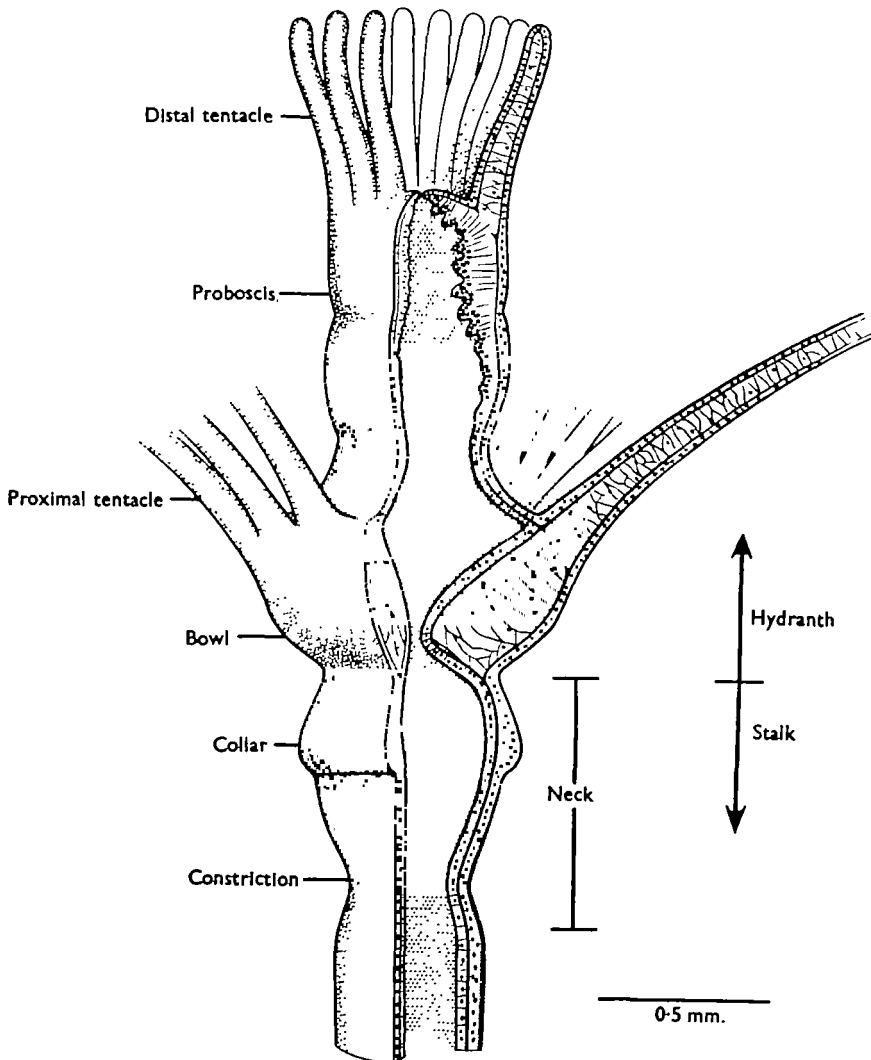

Fig. 1. A *Tubularia* polyp. The hatched line on the right represents the perisarc.

the neck. Most of the stalk is covered with a thin layer of perisarc. Immediately below the hydranth is a ring of stalk tissue which lacks perisarc. This will be termed 'the collar', and the remaining neck 'the covered neck'. The endodermal cushion at the base of the proximal tentacles divides the enteron into an oral and an aboral chamber and forms an effective valve between these chambers.

Portions of the behaviour of *Tubularia* have been described by Pearse (1906), Beutler (1925), Leghissa (1951) and Josephson (1961, 1962). The musculature of the polyp is confined to the hydranth and distal stalk. Longitudinal fibres run in the ectoderm of the neck, tentacles, proboscis and hypostome. Circular fibres run in the endoderm of the neck, proboscis and hypostome. With this effector system the animal can bend its tentacles orally or aborally, shorten and constrict its neck, open or close its mouth, pass a wave of peristalsis down the proboscis from apex to base, and constrict, bend or otherwise alter the shape of the proboscis, which is the most mobile part of the animal.

While some elements of the behaviour are simple, local movements, others consist of complex and variable sequences or combinations of movements representing the integrated activity of muscles over a wide area. Most active regions can give graded responses. Further, the shape and posture of a part at any given moment is influenced mechanically by activities elsewhere in the animal. For example, a sudden shift of fluid up the proboscis often causes the apex to dilate and the distal tentacles to sway inward as though in a muscular flexion.

Spontaneous activity

Much of the activity seen in unstimulated polyps consists of small, local movements: oral flexions of single proximal tentacles or groups of tentacles, oral and aboral flexions of single distal tentacles or groups of tentacles, occasional massed writhings of numbers of distal tentacles, lateral flexions of the proboscis together with concerted aboral flexions of the distal tentacles similar to movements made during feeding, and slight swaying or rotation of the hydranth upon its stalk through postural adjustments of the neck musculature. But the most striking spontaneous movements are the rhythmic activity cycles, here called 'concerts'. Hydranths of all ages and settled actinula larvae perform more or less regular activity cycles consisting basically of a pumping sequence during which fluids are shifted from one end of the proboscis cavity to the other or from the stalk cavity to that of the proboscis and back. Concerted oral flexions of the tentacles nearly always accompany the visceral pumping movements during the up-flow stage, hence the name 'concerts'. Many features of the concert as we will describe it here were first outlined by Beutler (1925). Her general conclusions concerning the role of the movements still appear valid and are echoed in our own.

In the ideal 'full concert' the movements begin with the hydranth in a relaxed position (Fig. 2a), the tentacles inclined outward, the neck and proboscis moderately extended. The proboscis wall just above the bases of the proximal tentacles contracts, so that there is a fluid shift toward the distal end of the cavity. At the same time the proximal and distal tentacles show a series of oral flexions which bring them together over the mouth region, and the neck contracts, also in a series of separate jerks, so that fluid is forced up through the neck valve and joins with fluid collected at the distal end of the proboscis cavity (Fig. 2b). The basal constriction of the proboscis wall

disappears in a few seconds, the whole organ becoming evenly dilated. A wave of peristalsis then passes down the proboscis from hypostome to base causing the fluid to flow back through the valve into the stalk. As it does so, the neck region swells and lengthens perceptibly (Fig. 2c) as the hydranth again resumes its resting position (Fig. 2d). The full concert undoubtedly serves to stir up the gastrovascular contents and to mix fluid from the stalk with fluid from the hydranth. The function of the tentacular contractions which accompany concerts is, at the moment, unclear. In sexually mature specimens the hollow, contractile racemes which bear the gonophores arise from the proboscis wall in the intertentacular region. At each concert fluid may be forced out into the racemes during the 'up' phase and again during the 'down' phase, these being the periods when the proboscis musculature is exerting pressure on the contained fluid mass. This tidal ebb and flow is detectable in the spadices of the gonophores and presumably serves to convey the products of extracellular digestion to the developing sex cells and larvae.
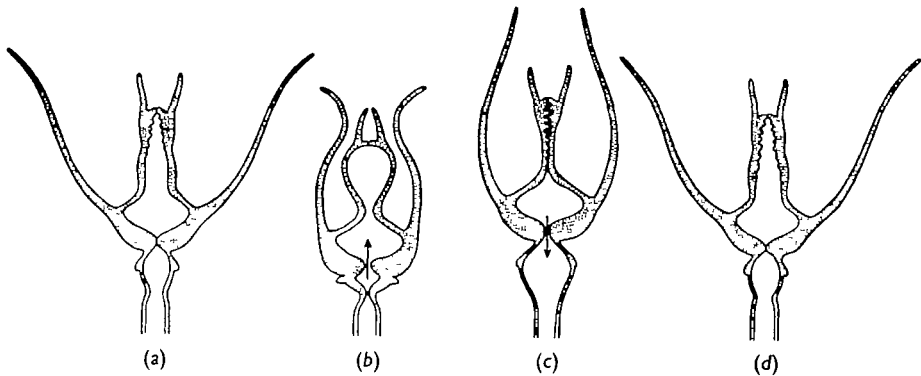

Fig. 2. A concert in a *Tubularia* polyp. The arrows in (b) and (c) indicate the direction of fluid movement through the opened neck valve.

Movement of fluid and particles in the stalk canal is brought about by the action of cilia lining this region as well as by the muscular contractions of the hydranth and neck. The stalk canal is subdivided into two or three longitudinal channels by lamellae projecting from the endoderm. Particles are conveyed along these channels by ciliary action. As Allman (1871) observed, the movement may be in opposite directions in adjacent channels.

The most common variation of the full concert is the concert in which the neck contractions do not occur or are so feeble that they are ineffective in forcing fluid up through the valve. In such cases the movement of fluid is confined to a shift within the proboscis cavity and racemes. Concerts with feeble or no neck components are commonly seen in animals which have not recently fed, or which have lost fluid from the gastrovascular cavity during egestion of wastes or through damage to the stalk. Occasionally they are seen in well-fed animals, interspersed among the full concerts. In starved animals the volume of fluid in the proboscis cavity may be so small that the initial contraction of the base of the proboscis is not seen at all, or is shown only indirectly by the slight dilation of the hypostomal end. Leghissa (1951) describes a form of incomplete concert similar to that described above, but one in which the neck

contraction comes in after the tentacular flexions have begun. This was frequently seen in our material as well. There are other variants of the concert. The proximal tentacles may travel to the oral position in a single sweep, instead of in a series of jerks. They may move only slightly or, rarely, not at all. Non-concert flexion of proximal tentacles is usually confined to the middle or terminal portions of the tentacles involved, while flexion during a concert begins at the base of the tentacles and only later do more peripheral portions also flex. The distal tentacles are often inactive during concerts.

In recently settled post-actinula larvae the proximal tentacles tend to shorten symmetrically rather than to bend during concerts and, when they bend, it may be either in the oral or in the aboral direction. The tips of the tentacles are more apt to bend aborally, the bases orally. When the neck contracts strongly, purely aboral flexions are often seen. The capacity to perform well-defined aboral flexions is almost completely lost after 10 days of settled existence. While exceptional hydranths in established colonies occasionally show aboral flexions of the tentacle tips, concert flexions in mature hydranths are normally purely oral. In the greater mobility of its tentacles the actinula larva somewhat resembles primitive tubularians such as *Euphysa* (Rees, 1957). The elaborate and specialized concerts of *Tubularia* and the comparable rhythmical behaviour patterns of *Corymorpha* (Parker, 1919), *Porpita* (Mackie, 1959) and certain sessile colonial hydroids (Beutler, 1925) probably evolved from simple, primarily digestive movements such as the peristaltic waves described by Beutler (1924) in *Hydra*.

The performance of a full concert in *Tubularia crocea* at 16° C. takes about 20 sec. The peristaltic wave begins 6–10 sec. after the onset of the concert. In concerts without neck contraction there is usually a delay between the termination of the tentacle flexion and the onset of peristalsis. This delay can be several seconds. Leghissa (1951) reports that a wrinkling of the ectoderm travels up the proboscis during this period. Concerts take place very regularly in isolated well-fed hydranths. In a normal, intact colony, where the hydranths are interconnected by living stolons, there is usually a marked degree of interaction, and individual rhythmicities become hard to determine. In the siphonophore *Nanomia* a rather similar interaction has been described between the gastrozooids and palpons (polyp members) during their collaborative rhythmic, digestive movements (Mackie & Boag, 1963).

At 15° C. several post-actinulae showed a cycle frequency close to 1 cyc./min. Larger hydranths usually show a lower frequency, down to one cycle every 2 min., at this temperature. At Woods Hole, with water temperatures of 19–22° C., we found typical frequencies of around one per 30 sec. in the larger hydranths. Leghissa reports intervals between concerts of 3–20 sec. in an unnamed species of *Tubularia* at Naples, which may indicate that the water temperature was rather high. From Beutler's data it appears that the cycle frequency is higher in *Tubularia* than in other sessile hydroids, the times for a full cycle ranging from 2 min. (*Laomedea*) to over 10 min. (*Eudendrium*). Fulton (1963) reports 10–30 min. cycles in *Cordylophora*.

Responses to stimuli

Mechanical or electrical stimuli to the stalk evoke a concerted aboral flexion of the distal tentacles. Relaxation following the aboral flexion appears to be passive. Aboral

flexion of the distal tentacles following stalk stimulation and the oral flexion during a concert both appear to be a result of contraction of longitudinal musculature in the tentacles, for in both cases the tentacles become concave in the direction of movement. This is interesting, for it indicates that the longitudinal musculature of the oral and aboral sides of the distal tentacles is activated through different conducting systems: the aboral musculature through the conducting system which runs through the stalk and hydranth, the oral musculature through the conducting system which co-ordinates the concerts.

The reactions of parts of the hydranths to local stimulation have been explored in detail by Leghissa (1951) and in general our results agree with his. However, where Leghissa reports that the proximal tentacles flex toward the side of the stimulus, we find, as did Pearse (1906), that they flex in the oral direction, whether the stimulus is on the oral, aboral or lateral side.

When a suitable food object, such as an *Artemia* nauplius, strikes a proximal tentacle, the tentacle flexes in the oral direction with the prey adhering to it. Then, after a pause, the distal tentacles open with a concerted aboral flexion, the proboscis bends over to the stimulated side, and the stimulated tentacle often bends over still further, bringing the prey against the mouth which has been exposed by the opening of the distal tentacles. A single electrical stimulus to a tentacle often evokes the same sequence of events. Mechanical stimuli, however, elicit only flexion of the oral tentacles, the degree of flexion varying with the stimulus strength. The fact that a brief electric shock to a tentacle evokes a whole sequence of responses while mechanical stimulation elicits only flexion of the tentacle suggests that stimuli other than mechanical stimuli are involved in the initiation of the normal feeding response.

In normal feeding the distal tentacles close around the prey as soon as they make contact with it. The proximal tentacle then pulls away, leaving the prey in the oral region. Rejection of unsuitable food occurs at this stage. The distal tentacles independently flex in the aboral direction, or writhe slowly, so detaching themselves from the prey, which eventually falls away. It is possible that the prolonged writhing of distal tentacles sometimes seen in unstimulated animals represents a rejection to some unknown chemical in the water. If the prey is acceptable, the mouth is pulled open by contraction of the longitudinal muscle fibres in the hypostome and proboscis, and the lips slowly creep around the edges of the prey, engulfing it. During the period when food is being ingested (a few seconds to several minutes) concerts are suppressed, emerging again when the lips have come together completely, or have closed around a narrow part of the prey. This recalls the observation that spontaneous contractions of *Hydra* are temporarily eliminated during feeding, or in response to reduced glutathione (Rushforth, Krohn & Brown, 1964).

II. *Electrical activity in the polyp*

General features of the electrical activity

It is now known that the spontaneous potentials earlier described (Josephson, 1962) are only part of the spontaneous electrical activity produced by a *Tubularia* polyp. In addition to these potentials, found principally in the neck, pulses of a different nature can be recorded from the distal hydranth and from the tentacles.

Spontaneous potentials from a polyp, excluding the tentacles, appear to be referable to one or the other of two pacemaker systems, one located principally in the neck, the

other located principally in the hydranth. This localization is based on the size of the recorded potentials in different polyp regions. There are two classes of pulses (Figs. 3, 4). Pulses of one class are large in the neck and greatly attenuated in the hydranth or in the more proximal stalk. These potentials will be called neck pulses (NP's). The potentials of the second class are large in the proboscis, but usually can be recorded as smaller potentials in the distal stalk as well. These will be termed 'hydranth pulses' (HP's). Potentials from the subtentacular bowl region of the polyp sometimes do not fall cleanly into either of these classes and are discussed below.
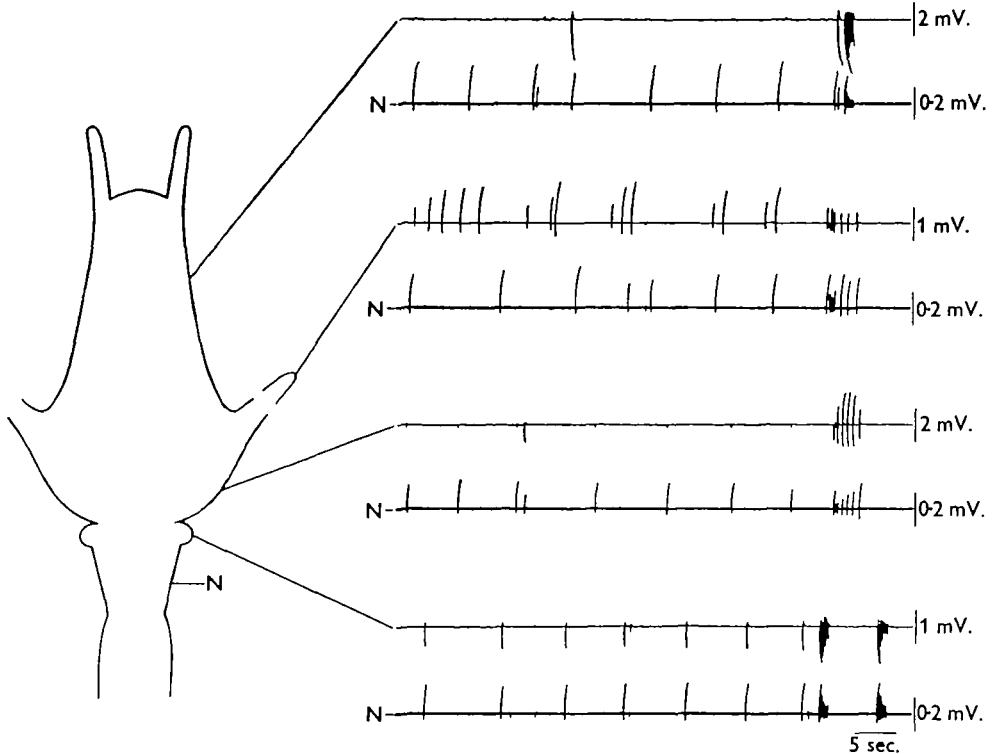

Fig. 3. Spontaneous activity recorded with suction electrodes on different polyp regions. The records were collected in pairs, in each case activity from the point indicated is compared with that recorded simultaneously from the neck. The upper and lower record pairs were collected first and the burst pattern had changed somewhat before the two middle pairs were recorded. A negative potential is an upward deflexion in this and the following figures. (Retouched for reproduction.)

The neck system in the distal stalk and the hydranth system in the proboscis are unitary systems with respect to the distribution of potentials. HP's appear nearly simultaneously from each of two electrodes anywhere on the proboscis and an HP is never seen from just one of the pair (Fig. 5). Similarly NP activity throughout the neck is everywhere nearly coincidental. But NP's and HP's at any site are not all-or-nothing events. The amplitude and often also the shape of the pulses are influenced by previous activity. Such changes can be quite local; for example, two successive HP's may show facilitation at one recording site and antifacilitation at an adjacent site.

HP's can occur independently or in conjunction with NP's during NP bursts.

Autonomous HP's appear as single pulses or as short bursts, usually of two to four pulses (Fig. 4). HP's during NP bursts occur in either a one-to-one relation to NP's or sometimes, especially early in NP bursts, as short bursts of two or three HP's for each NP (Figs. 6, 7). Some NP bursts have no associated HP activity. Purely NP
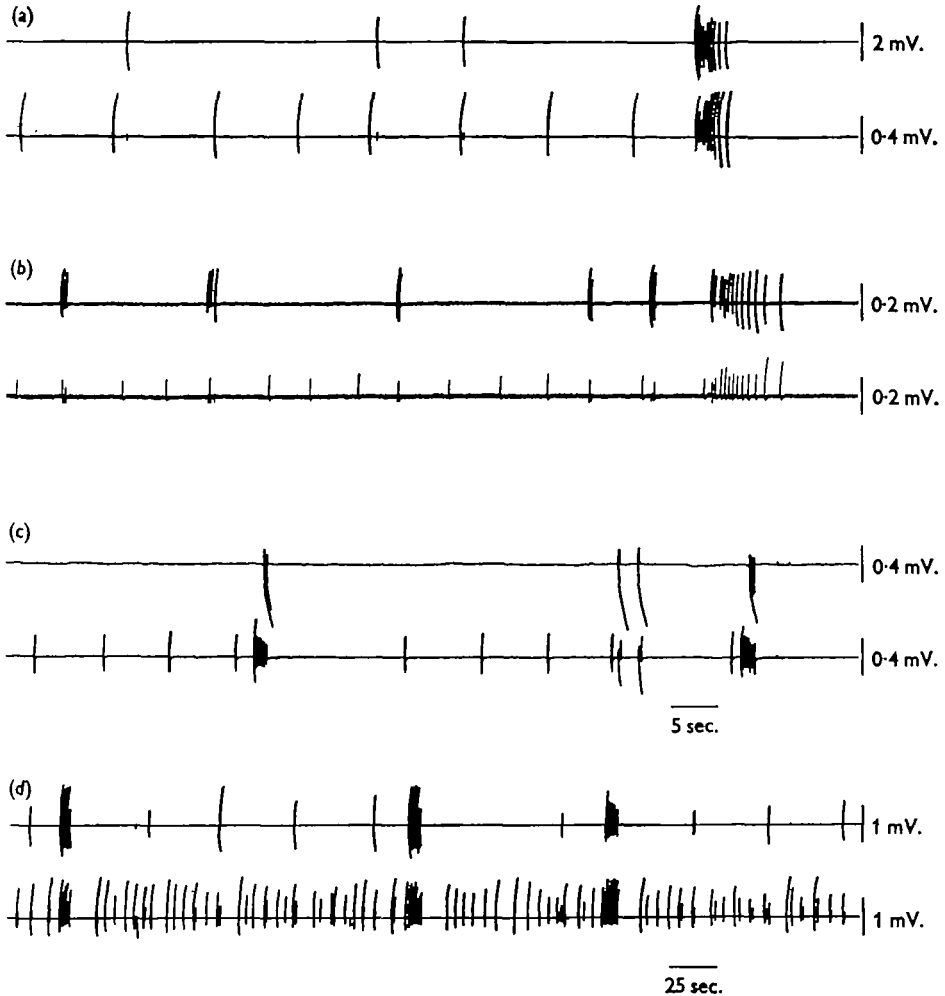

Fig. 4. Activity patterns in the HP and NP systems. The recordings were made with two metal electrodes, one in the proboscis (upper trace of each pair) the other in the polyp neck (lower trace). The upper traces show principally HP's, the lower traces NP's (note in (c) that HP's in the lower trace are sometimes larger than are the NP's). Each record pair is from a different polyp. (Retouched for reproduction.)

bursts are rare in unstimulated animals but are common during feeding when the mouth is open and food is being ingested. Infrequently HP activity begins in a burst before NP's are seen (Fig. 6). The most common pattern, however, is for the NP burst to begin and several NP's to be produced before the HP's also begin to appear (Figs. 4c, 7, 10). Bursts of this type are recorded in the proboscis as a series of small NP's, sometimes facilitating slightly, which are suddenly obscured by the appearance of the much larger HP's.

There are several reasons for believing that the HP's during NP bursts, especially during the later portions of the bursts, are driven by NP activity. (1) The pulse-burst pattern seems inherent to the NP system, and it is seen even in decapitated stalks (Josephson, 1962). NP bursts can occur without there being a corresponding HP component, and the pattern of NP pulses within the burst is not noticeably changed when the HP system begins to respond. There is no indication from the pulse pattern in the bursts that the HP system, when activated, itself takes over the
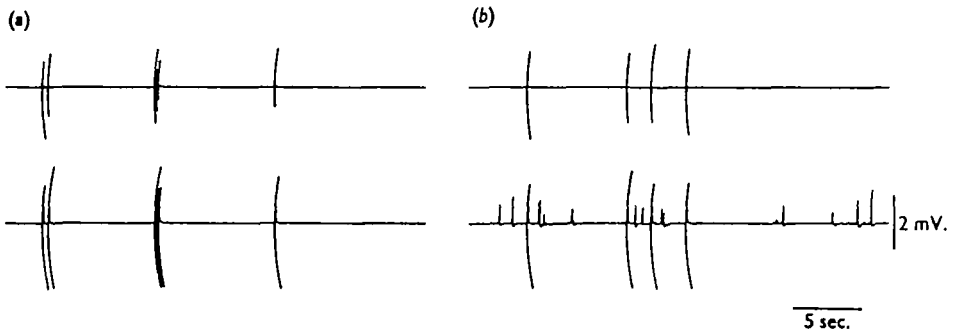

Fig. 5. (a) Potentials recorded simultaneously from two suction electrodes on the proboscis. (b) As (a) after the suction of the electrode giving the lower trace had been increased until 'suction potentials' began to appear.
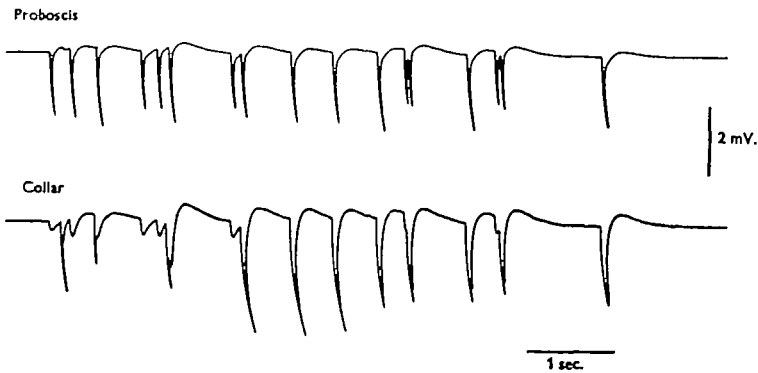

Fig. 6. An NP–HP burst which began with HP activity. Recorded from collar and proboscis with suction electrodes.

function of pacemaker. (2) It is possible to trigger NP's by stalk stimulation (Josephson, 1965). With repetitive stimulation the NP system can be driven to give pulses resembling the usual NP bursts. The HP system responds during these pseudo-bursts just as it does during normal bursts; usually after a few NP's the HP system begins to fire in coincidence with the NP's (Fig. 7). Thus it appears that repetitive activation of the NP system, whether endogenous or due to external stimulation, leads to HP firing. (3) During bursts the onset of a pulse in the distal stalk usually precedes the onset of the corresponding pulse in the proboscis. The interval is not constant, however, and during portions of the burst the pulses in the proboscis can arise nearly synchronously with or can slightly lead those in the neck (Fig. 8).

Electrical activity of different polyp regions

The following is a summary of the types of spontaneous electrical potentials recorded from different polyp areas. The strictly local potentials which are recorded with suction electrodes and which appear to be a result of the suction are not discussed.
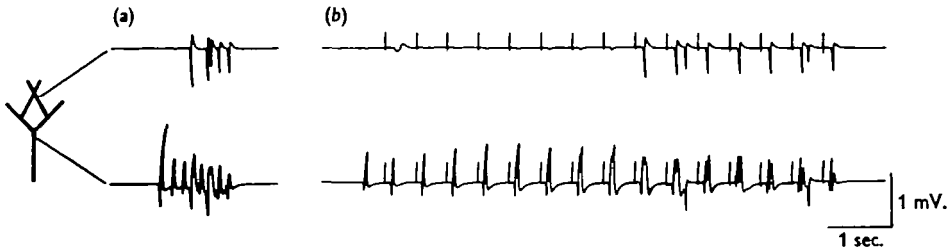

Fig. 7. (*a*) A spontaneous burst recorded from neck and proboscis with metal electrodes. (*b*) A pseudo-burst from the same preparation produced by stalk stimulation at two shocks per second. Note that the HP system sometimes fires twice following a single stimulus. The response in the proboscis following the first shock is the potential associated with opening of the distal tentacles (see Josephson, 1965).
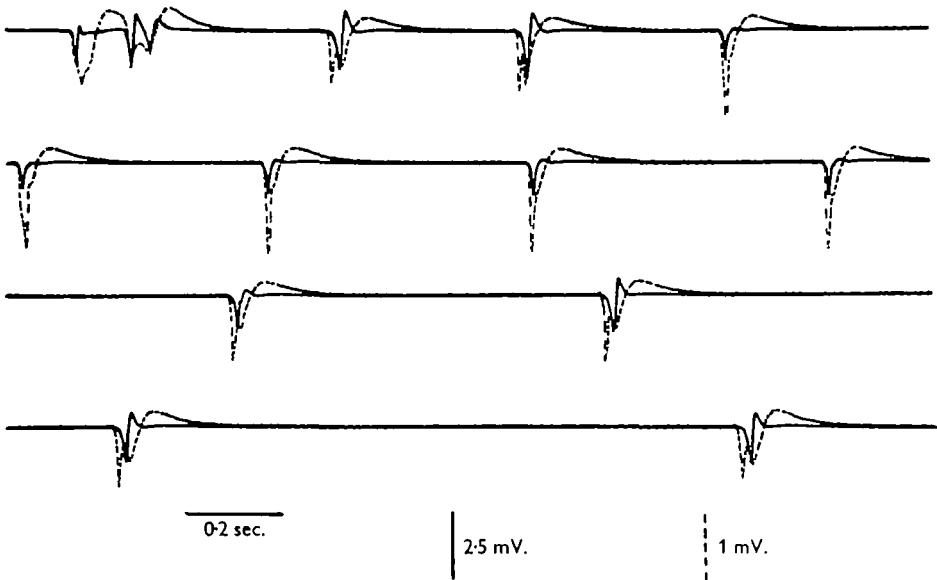

Fig. 8. A burst recorded simultaneously from collar (broken trace) and proboscis (continuous trace) with suction electrodes. Note the changing latencies between the onset of pulses from the two recording channels. (Retouched for reproduction.)

Amplitudes given are from maximum negativity to maximum positivity. The numbers in brackets indicate the number of different polyps from which recordings at each site were made. It should be emphasized that the spontaneous potentials are quite unlike the potentials which can be recorded throughout the polyp following stalk stimulation (Josephson, 1965). The latter are considerably smaller and shorter than the spontaneous potentials except in the stalk below the collar, where the spontaneous potentials can become very small or unrecordable.

(a) Covered neck

Metal electrodes (> 100). NP's vary considerably with electrode position. They are usually 100–500 μV., but range from barely detectible (10–20 μV.) to several mV. The NP's are usually complex, and contain both positive and negative components. The amplitude and shape of NP's are often strikingly changed by antecedent activity, especially during bursts. The NP duration ranges from about 20–100 msec. HP's range from barely detectable to 500 μV., and are usually less than 100 μV. They can be larger than the NP's. HP's begin with a positive deflexion which is followed by a slower and smaller negative tail, at least part of which is due to capacitative coupling to the amplifier.

Suction electrodes (15). It is somewhat difficult to use suction electrodes on the covered neck as they do not adhere as well here as on the more distal hydranth, probably because of the perisarc layer. NP's recorded from the covered neck with suction electrodes are smaller but less complex than those recorded with metal electrodes. They consist of a dominant negative spike followed by a small positive component which may be a result of capacitative coupling. Occasionally the negative spike is preceded by a small positive potential. The negative spike is usually 100–200 μV. but can be as large as 700 μV. Its duration is 20–40 msec. The NP's usually decline early in a burst but often begin to increase in amplitude again later in the burst when the pulse frequency is lower. HP's are 50 μV. or less and, as with metal electrodes, positive.

Fig. 9. Potentials recorded simultaneously from the covered neck (upper trace) and collar. The first and last pulses are NP's, the middle two are HP's. The collar trace was interrupted approximately one thousand times per second.

(b) Collar (12)

Only suction electrodes have been used to record from the collar. NP's are large, 100 μV. to 3 mV., and usually predominantly positive, often nearly monophasic with a spike-like positive potential lasting 15–80 msec. (Fig. 9). NP's usually become more complex in bursts but this may be due in part to the addition of HP components. HP's are 100–500 μV. and positive. In some preparations HP's from the collar are larger than NP's.

(c) Proboscis

Metal electrodes (15). HP's range from less than 300 μV. to more than 3 mV. and usually have both positive and negative components. Their duration ranges from less than 20 msec. to 60 msec. or more. HP's often appear in short bursts, sometimes with

an interval of less than 50 msec. between successive pulse peaks. When one HP follows another closely it can arise from the falling phase of the preceding pulse. NP's are less than 100 μV. and consist of a postive potential followed by a smaller negative tail.

Suction electrodes (21). HP's sometimes contain both positive and negative components but are often nearly monophasic and positive. Their amplitude can be as great as 5 mV. but is more often about 1 mV. The HP duration is on the order of 20–60 msec. NP's are principally positive and less than 100 μV.

A suction electrode was slipped through the mouth of one animal and recordings were made from somewhere inside the proboscis. The HP's were 2 mV. positive potentials followed by slower 1 mV. negative potentials, very much like the pulses recorded from the outside surface of the proboscis in several animals, and the pattern of activity was identical to that recorded simultaneously from an electrode on the external surface.

(d) Subtentacular bowl

Metal electrodes (3). Potentials from the bowl were like those from the proboscis. HP's were large, 300 μV. to 2 mV. Single HP's were predominantly positive, but
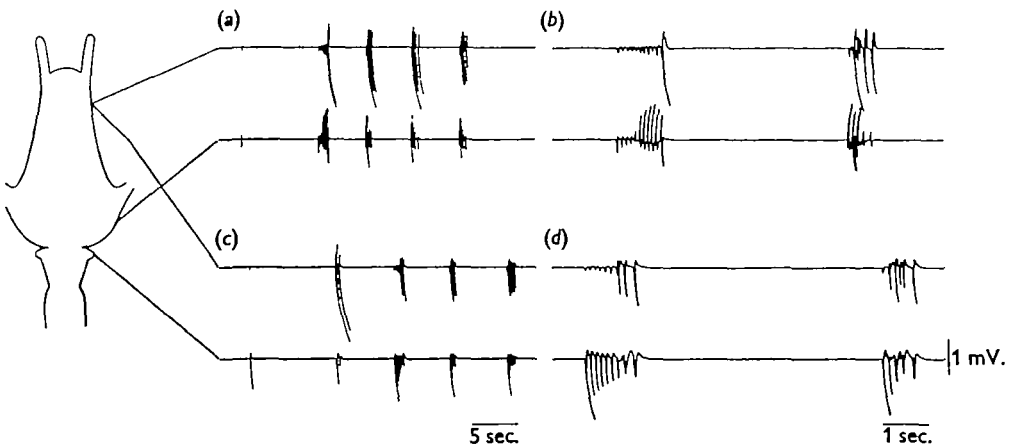

Fig. 10. Progressive invasion of the subtentacular bowl and proboscis during an NP burst. (a) A series of spontaneous bursts recorded simultaneously from the proboscis and bowl with suction electrodes. (b) The first two bursts of a similar series recorded somewhat later and at greater speed. (c) and (d) as (a) and (b), recorded from the proboscis and collar. Note that for the first burst of a series the bowl begins producing large pulses only after some number of NP's have been fired, and that the proboscis begins to respond still later. (Retouched for reproduction.)

during bursts the HP's became predominantly negative or very complex, sometimes with two positive and two negative peaks. NP's were small, 20–200 μV., and positive or biphasic. In one animal the proboscis was transected about half-way between the distal and proximal tentacles. HP's continued to be recorded in the bowl and neck.

Suction electrodes (6). NP's range from 20–300 μV. and are usually positive. Single HP's are 200–1250 μV. and also predominantly positive. During bursts the potentials from the bowl often become larger (up to 2 mV.) and more complex. Small potentials, less than 100 μV., are often recorded from the bowl. These occur with greater frequency than either NP's or HP's, and may represent pick-up from adjacent tentacles.

Records were made simultaneously from the bowl and proboscis in four animals. In two of these both the bowl and proboscis began producing large pulses (HP's) on the same NP during NP bursts. In the other two animals the bowl was the first to respond and often would produce several large pulses before the proboscis also began to follow in the burst (Fig. 10). These results indicate that NP activation of the hydranth can be progressive, first affecting the bowl and triggering the HP system in the proboscis only on later pulses of the NP burst. And in some cases the pulse-producing system of the bowl, or at least of part of the bowl, is not a part of either the HP or NP systems, but rather a separate system interposed between these two.

(e) *Proximal tentacles* (10).

Only suction electrodes have been used to record from proximal tentacles. A new class of potentials, termed tentacle pulses (TP's), is encountered in the tentacles. TP's
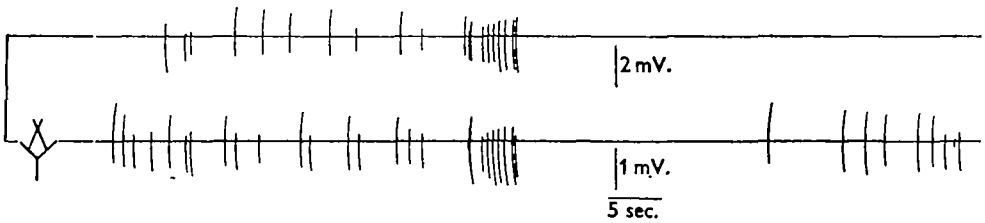

Fig. 11. Spontaneous activity recorded simultaneously from two tentacles with suction electrodes.
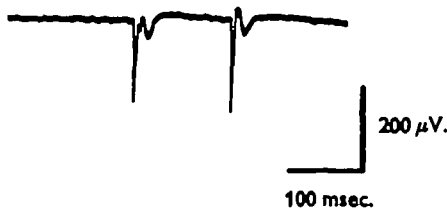

Fig. 12. A pair of pulses recorded from an isolated proximal tentacle with a suction electrode.

range from about 50 μV. to several mV. They are generally short, 50 msec. or less, and frequently contain an initial spike-like component whose duration is well under 10 msec. TP's recorded from a single site often vary considerably in amplitude and shape (Fig. 11). They appear irregularly, usually with a frequency greater than that of either HP's or NP's, and often as pairs of pulses separated by 60–500 msec. During HP bursts the TP's also appear in bursts. Single HP's or the first HP of a burst are usually seen in the tentacles as positive potentials, 250 μV. or less, and are sometimes not recorded at all. Subsequent HP's are typically associated with potentials in the tentacles which are the size of the usual TP's or which facilitate to reach TP amplitude. Activity in different tentacles of a hydranth is asynchronous except during HP bursts (Fig. 11). NP's are often not seen in the tentacles, and when recorded they are 100 μV. or less and positive. Records made from one isolated tentacle gave only short TP's, either singly or in pairs (Fig. 12); neither TP bursts nor anything that could be construed to be either HP's or NP's were seen.

Spread of individual pulses in the polyp

Some two-site recordings have been made at recording speeds allowing temporal resolution down to 2–5 msec. The interpretation of these records is rather uncertain. The pulses usually do not begin abruptly, thus determination of their time of origin may be somewhat inaccurate. The pulse shapes are frequently different at two sites, so it is not possible to measure time differences between corresponding points on the pulses as the corresponding points are not known. Further, time delays between two sites cannot be converted into conduction velocities for neither the site of pulse origin nor the pathways along which it propagates are known.

Two general features have emerged from analysis of these records:

(1) The latency between homologous pulses at two sites can be quite variable. In four animals NP's were recorded simultaneously from an electrode on the collar and one on the covered neck distal to the constriction. In two of these some of the NP's appeared first in the neck and some appeared first in the collar, with the interval between the onset of NP's at the two sites being as great as 8–10 msec. Similar variability was seen in the latencies between TP's recorded from two tentacles during bursts. In the two animals from which such recordings were made TP's sometimes appeared first in one, sometimes first in the other tentacle, with latencies between TP onset at the two sites being sometimes greater than 10 msec. On the other hand HP's recorded from two electrodes on the proboscis did not show variable latencies. In four animals HP's always began nearly simultaneously at the two sites. In a fifth animal HP's consistently began first near the base of the proboscis with the HP onset coming 4–6 msec. later from an electrode near the base of the distal tentacles.

(2) The latency between NP's and HP's in a burst is also not constant, but varies during the course of the burst. Commonly NP's in the neck or collar lead HP's in the bowl or proboscis during the initial portion of a burst, but the NP to HP interval shortens during the early part of the burst and lengthens again during the later part so that for the middle pulses the NP's and HP's are nearly synchronous (Fig. 8) or sometimes the HP's slightly lead the NP's.

There are several possible explanations for changing latencies between pulses recorded at two sites. There may be not a single pacemaker but rather a number of pacemakers which can initiate pulses. The effective pacemaker may be sometimes closer to one, sometimes closer to the other recording electrode. Or there may be changes in the conduction velocities of different pathways involved, due to facilitation or fatigue, such that the transit time between pacemaker firing and pulse production varies in different ways for the two recording sites. Or it is possible that there is an unseen signal which triggers pulses over a wide area, and the variability is due to variability in the latency between arrival of this signal and pulse production at any point. Of these possibilities, the concept of unfixed and changing pacemaker loci seems most probable.

III. *The relation between electrical activity and behaviour*

An earlier paper (Josephson, 1962) attempted to relate polyp behaviour and electrical activity recorded from the distal stalk (i.e. the NP system). The correlation was rather unsatisfactory. Concerted elevation of the proximal tentacles was reported to occur in association with NP bursts, but not all bursts had associated tentacle activity. Two

factors have somewhat clarified the relation between electrical activity and behaviour. (1) It is now realized that concerts occur in a wide range of graded intensities. Previously only the more vigorous concerts were recognized, weaker ones were thought to be chance coincidence of individual tentacle movements in a number of tenctacles. It is now known that concerts, as they have been here defined, may involve only a slight twitch of the tentacles; and that even weak concerts may be distinguished from individual tentacle movements by (a) the following peristaltic wave, and (b) the tentacle movement beginning at the bases rather than along the lengths of the proximal tentacles. Further, even in weak concerts there is often a concerted elevation of the distal tentacles accompanying that of the proximal tentacles. (2) The use of flexibly mounted metal electrodes and especially suction electrodes has revealed the presence of the HP system, which appears to be directly related to concert activity.

Concerted tentacle elevation is quantitatively correlated with HP's. Brief, twitch-like tentacle concerts generally appear with one or a few HP's, vigorous tentacle concerts with longer HP bursts. The exact relation between HP's and concerts varies somewhat from animal to animal. In some polyps quite pronounced tentacle movements appear with single HP's, in other animals the tentacle movement with single HP's is slight. But after an animal has been observed for a while and 'calibrated' it is possible to describe quite accurately the concerted activity of the tentacles by observing a recording channel displaying the HP's. The partial correlation between tentacle concerts and NP bursts finds its explanation in the loose coupling between the HP and NP systems. Some NP bursts do not excite the HP system and there is no concerted tentacle activity. The HP system fires spontaneously in single pulses and short bursts, so there are concerts, although usually weak ones, without NP activity. The longer HP bursts are those which appear in coincidence with NP bursts, so the more vigorous concerts generally occur during NP bursts.

The relation between electrical activity and the neck shortening leading to tidal exchange is still not clear. Neck shortening occurs during some NP bursts when both the NP and HP systems are active. Short NP–HP bursts, containing for example 3–5 pulses, are usually associated with no appreciable neck shortening while longer bursts, containing five or more NP's and HP's, generally have an associated neck-shortening component.

Some evidence on the control of neck shortening has been obtained from observations of polyp behaviour while the NP system was driven by stalk stimulation to produce pseudo-bursts. Typically in a pseudo-burst a variable number of NP's are produced before the HP system also begins to respond. In a number of polyps the stalk was stimulated at frequencies of 1–5/sec. and the duration of the stimulus burst varied so that sometimes the HP system was activated and sometimes not. The results with each polyp were the same. During that portion of the pseudo-burst in which only the NP system was active there was slow straightening of the hydranth on the stalk, indicating some contraction of longitudinal musculature in the neck. It was possible to trigger as many as ten NP's without exciting the HP system, in which case there was only slow neck straightening. But if the stimulus burst was long enough to excite just one HP there was a sudden, vigorous neck shortening of the type leading to tidal exchange. The neck shortening occurred in coincidence with the HP. As few as three or four NP's plus one HP gave pronounced shortening. This result is surprising, for

single HP's or short HP bursts do not lead to neck shortening when these occur independently and not in conjunction with NP's. These results indicate that the NP system alone can evoke slow neck responses. In fact, in one animal observed there was slight but noticeable neck shortening with each spontaneous NP. There would seem to be several possible explanations for the observation that a single HP coming after a number of NP's in a pseudo-burst gives vigorous neck shortening while single HP's occurring spontaneously do not. The HP and NP systems may act synergistically to control neck shortening, a number of NP's being required to condition the neck musculature so that it responds to HP's. Or, perhaps more likely, there may be portions of the HP system which control neck shortening and which are activated immediately during pseudo-bursts but which are invaded only after some number of HP's have been fired in spontaneous bursts.

As stated earlier, concerts in recently fed, distended animals are of long duration, vigorous, and generally with pronounced neck contraction and tidal exchange; while brief concerts, often with no neck response, are interspersed among the more vigorous concerts in starved animals or in animals which have lost fluid from the enteron. These changes in behaviour are reflected in changes in the electrical activity patterns of the HP and NP systems. Almost all the electrical activity in well-fed animals consists of regular NP–HP bursts of long duration with single NP's during the intervals between bursts. In empty animals HP bursts and short NP–HP bursts are seen, as well as longer NP–HP bursts. and there is much less regularity in the overall pattern.

The relation between tentacle pulses and tentacle movements is at present not understood. Some TP's are associated with flexion of the tentacle from which the recording is made, others appear to be associated with movements of adjacent tentacles. The only uncomplicated case was that in which recordings were made from a single, isolated tentacle. Each pulse or pair of pulses recorded was associated with a tentacle twitch in what would have been the oral direction had the tentacle still been attached.

IV. *Interaction between polyp pacemaker systems*

Interaction between the HP and NP systems

(a) *Interaction during bursts.* Evidence has been presented above which indicates that the HP system is usually driven by the NP system when the latter fires in a burst.

(b) *Single pulse interaction.* A number of recordings were analysed in the following manner to see if there is interaction between single HP's and NP's as well as interaction during NP bursts. These were two-channel recordings, one channel displaying HP's from the proboscis and the other displaying NP's from the distal stalk. A single HP was defined as an HP separated from preceding and following HP's by at least 1 sec. The onset of single HP's in these records were used as zero time reference points (see Fig. 13). For each single HP a second reference point was selected, one coming 0–25 sec. after the HP onset. The interval between the HP and the second reference point was taken from a random number table using 1 sec. increments. Four intervals on the records were measured using these two reference points: the time between the HP and the preceding NP (A); the time between the HP and the following NP (B); the time between the randomly selected point and the preceding NP (C); and the time between the randomly selected point and the following NP (D). If a second HP

occurred during any of these intervals, that interval was not included in the final tabulation. The intervals were measured in 200 msec. increments, the first increment being 100 msec. on either side of the reference points. Thus the interval ranges A and B share one increment, that about the reference point, as do interval ranges C and D. If a reference point and an NP coincided so closely that it could not be determined which came first (from pen-writer records generally at 5 mm./sec.), an entry was made for the first increment of either A or C, whichever was appropriate, but not for both A and B or C and D. Occasionally an NP burst triggers but a single HP, this coming in coincidence with the terminal NP of the burst. Such HP's appear to be a result of NP burst activity rather than single pulse interaction and were not used as reference points. Using only single HP's as defined above eliminates many HP's in the records from consideration, for many HP's come in short HP bursts, but it also eliminates the problem of having to decide which HP of several in a burst has affected the probability of NP firing.

Fig. 13 shows the frequency histograms of the measured intervals from almost 6 hr. of records collected from twenty-four different polyps. The lower histogram, that measured with the randomly chosen reference points, gives the distribution which would be expected for the HP–NP intervals were there no HP–NP interaction, that is, if HP's and NP's occurred randomly with respect to one another. The actual HP–NP interval distribution (upper histogram) is quite different, there being a large peak about the HP reference point. The tendency for values to congregate near the zero time in the A range indicates that the probability of HP firing is greater immediately after an NP has fired than at longer intervals after NP firing. This is perhaps to be expected from the HP triggering by NP's during NP bursts. But the peak in the distribution comes in the B range to the right of the reference time, indicating that the probability of NP firing is greater immediately after HP firing than it is several seconds after a single HP. There are several possible explanations for the tendency of NP's to occur either immediately before or immediately after a single HP. For example, the NP and HP systems might both be occasionally driven by a third system which causes them to fire together but with a variable delay between NP and HP onset. But the simplest hypothesis is that there is direct interaction between the two systems, HP firing sometimes triggering the NP system to fire within the following few hundred milliseconds, and similarly NP firing sometimes triggering the HP system. It is this hypothesis which will be assumed in the following discussion.

The interval between an HP and an NP which it triggers can be quite long, up to 300–500 msec. (Fig. 13). This means that a triggered NP can begin after the electrical events of the triggering HP have apparently subsided. It is not known what occurs during the quiescent period between the completion of an HP and the onset of a triggered NP.

A stimulus to the stalk is most effective in triggering an NP if it comes shortly after either a spontaneous or a triggered NP (see below). The records analysed for the HP–NP interval histogram were re-examined to see if there is a similar sensitive period in the NP cycle for NP triggering by single HP's. A triggered NP was defined as one coming 500 msec. or less after an HP. This interval includes most of the peak in the B range of Fig. 13. The time between each single HP and the preceding NP was measured, and it was noted if that HP did or did not trigger an NP. The results of this

analysis are presented in Fig. 14. It can be seen that an HP is not equally likely to trigger an NP at all times in the NP cycle. The probability that an HP will trigger an NP is about 0·3 if the HP comes within the first second after a spontaneous NP. For
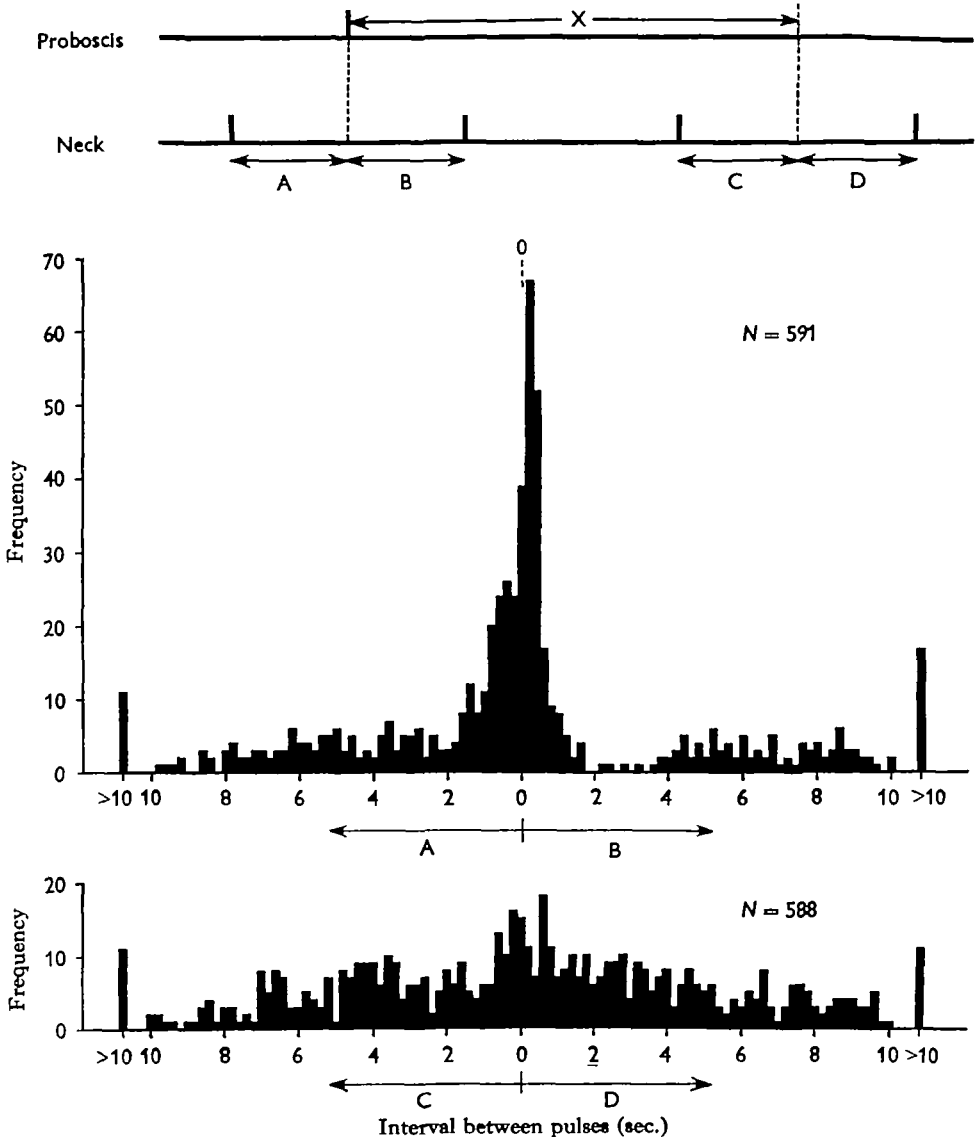

Fig. 13. Time-intervals between firing of single HP's and NP's. X is a time interval between 0 and 25 sec. taken from a random number table. A new value for X was used with each single HP. Thus the lower histogram gives the HP–NP interval distribution which would be expected were there no HP–NP interaction; the upper histogram gives the HP–NP interval distribution actually found. Further explanation in text.

longer intervals this probability at first drops, but then rises to a maximum late in the NP cycle. All twenty-two of the single HP's which came eight or more seconds after the last NP triggered NP's. Thus the NP system is most sensitive to triggering by single HP's either just after it has fired or just before it is due to fire again.

From inspection of these records it is clear that at least those HP's coming late in the NP cycle reset the NP pacemaker when they trigger NP's, or in some other way make the NP pacemaker temporarily incapable of initiating an NP. The intervals between triggered NP's and the following spontaneous NP's are approximately the same as the intervals between spontaneous NP's. Were the NP pacemaker not reset or made temporarily ineffective, the interval between a triggered NP and the following spontaneous NP should be very short when the triggering HP comes late in the NP cycle, for such HP's are occurring shortly before the expected time of NP pacemaker firing.
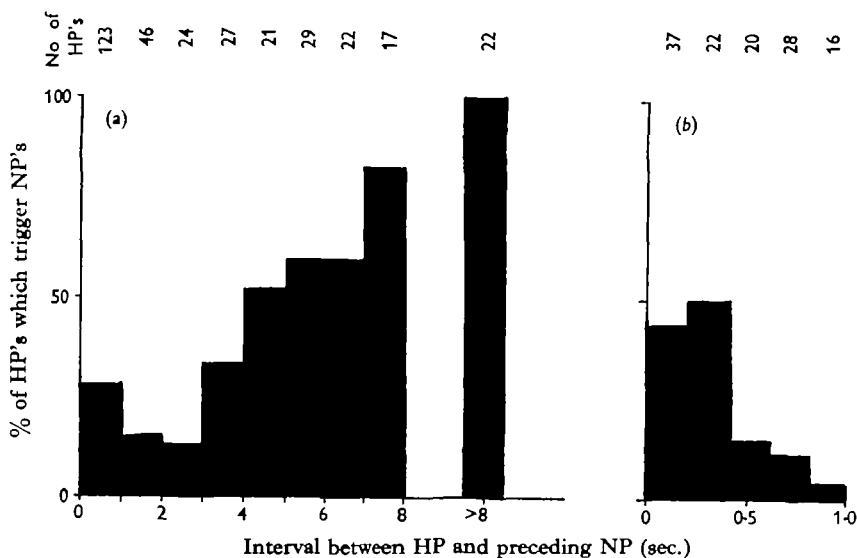

Fig. 14. The proportion of single HP's which triggered NP's as a function of the intervals between the HP's and the preceding NP's. (b) An expansion of the 0–1 sec. portion of (a). Further explanation in text.

The sensitivity of the NP system to triggering late in the NP cycle is probably responsible for the dip in the histogram of Fig. 13 in the 1·7–3·7 sec. portion of the B range. This area would have been filled by HP's coming late in the NP cycle, 1·7–3·7 sec. before the NP system was due to fire. But such HP's usually trigger NP's, causing them to come prematurely and leaving a depopulated range in the distribution.

Proximal tentacle pacemakers and the NP and HP systems

Sporadic oral flexions of single proximal tentacles or small groups of tentacles are frequent during the periods between concerts of *Tubularia* polyps, and irregular pulses of variable amplitude (tentacle pulses—TP's) can be recorded from proximal tentacles during these interconcert periods. TP activity patterns from different tentacles are asynchronous between concerts, and only some of the pulses are correlated with visible movement of the tentacle from which the recording is made. These spontaneous movements and potentials indicate the presence of a number of pacemakers controlling the activities of individual proximal tentacles or tentacle groups.

During HP bursts the proximal tentacles are synchronously elevated, and TP's in all proximal tentacles appear in coincidence with the HP's. Thus it appears that the

HP system can drive the tentacle systems. Evidence was presented above that the NP pacemaker is the primary pacemaker for the longer bursts in the polyp, those in which both the HP and NP systems are usually active and during which there is especially vigorous concerted elevation of the proximal tentacles. TP's appear in coincidence with NP's during these bursts, but they are probably not driven directly by the NP system as are the HP's but rather indirectly through the HP system. If there is an NP burst which does not excite the HP system, tentacles do not show concerted elevation nor do TP's begin appearing in coincidence with the NP's. Vigorous concerts are usually followed by a period of tentacle quiescence during which there is little tentacle movement and the frequency of TP's is markedly reduced (Fig. 11). This indicates that NP-HP bursts affect the tentacle pacemakers, supressing their activity for some time following the bursts.

Oral flexion of single proximal tentacles or groups of tentacles may occur either more frequently or less frequently than do NP's during the interconcert period. If a polyp is observed while NP's are monitored with a loud-speaker it often appears that unconcerted proximal tentacle flexions are temporally associated with NP's more often than could be expected by chance. It has not yet been possible to verify such coupling by observation or by electrical recording from single tentacles. But a *Tubularia* polyp of the species and size used has 15–20 proximal tentacles, so there could be good correspondence between NP's and flicks of any of the proximal tentacles without there being obvious correspondence between NP's and the activity of one selected tentacle.

V. *Triggering the NP system by stalk stimulation*

Activation of the conducting system in the stalk termed the triggering system (TS) can trigger NP's in a polyp. The effectiveness of stalk stimulation in triggering NP's varies somewhat from polyp to polyp. In some polyps almost every stimulus above a threshold value triggers the NP system to fire. In most polyps, however, stalk stimuli usually do not trigger NP's unless they occur within a few seconds after NP firing. The following experiment was performed to characterize this sensitive period in the NP cycle. The stalk of a polyp was stimulated regularly at a frequency of one shock per 10 sec. Considered over long periods the stimuli arrived more or less randomly within the NP cycle. The stimulus intensity was somewhat above threshold for both the TS and DOS conducting systems in the stalk. The interval between each stimulus and the preceding NP was measured, and it was noted if that stimulus did or did not trigger an NP. Cases where the NP preceding the stimulus was either a triggered NP or one of the NP's of an NP burst were not included in the final tabulation. The results of this analysis are shown in Fig. 15, which represents about 106 min. of recording from one polyp, 75 min. from another polyp, and 5 min. periods from each of four additional polyps. Fewer than 50% of the stimuli triggered NP's with each of these animals. The histograms for the two animals with the longer records were quite similar when plotted individually, so grouping the data from a number of animals seems justifiable.

It can be seen from Fig. 15*a* that stimuli given within the first 3 sec. after a spontaneous NP are much more effective in triggering NP's than those coming later. Stimuli given in the interval 1–2 sec. are most effective, and are even more effective than those given 0–1 sec. after an NP. Fig. 15*b*, which is an expansion of the 0–1 sec.

interval, shows that the depression of trigger effectiveness is confined to the first 200 msec.; thereafter the proportion of stimuli which trigger NP's is as great as it is during the period 1–2 sec. It should be pointed out that there is a delay between a stalk stimulus and the arrival of TS activity in the hydranth. In these experiments the stimulating electrodes were 1·0–1·5 cm. below the hydranth. The conduction velocity of the TS is about 17 cm./sec., so TS activity did not arrive at the neck until 60–90 msec. after the stimulus. This means that with respect to the NP system the measured intervals occur slightly later in the NP cycle than is indicated in Fig. 15, which gives the intervals with respect to the time of stalk stimulation. It also means that the period of depressed triggering effectiveness, which presumably represents a refractory period, may be slightly longer than is indicated in Fig. 15b.
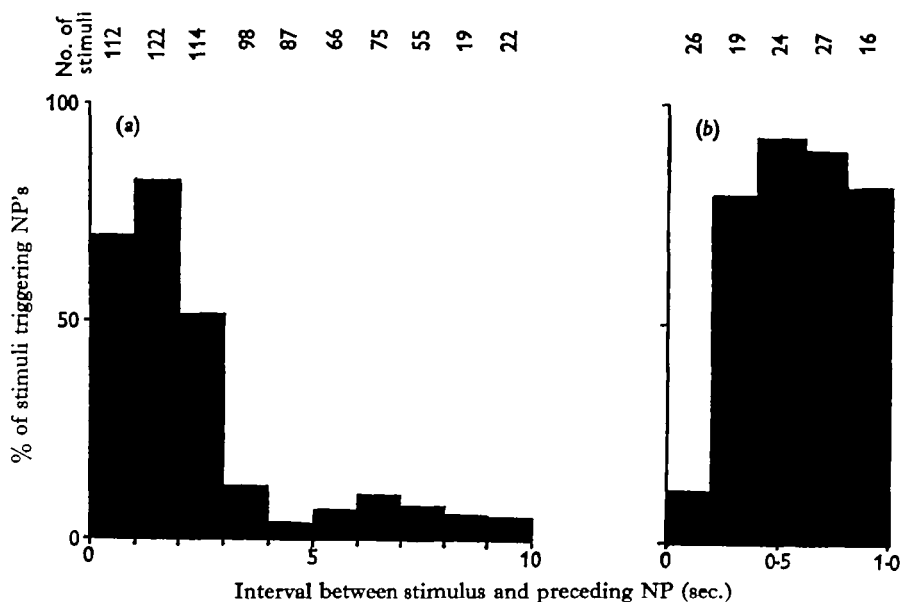

Fig. 15. The proportion of stalk stimuli which triggered NP's as a function of the intervals between the stimuli and the preceding NP's. (b) is an expansion of the 0–1 sec. portion of (a). Further explanation in text.

The NP system is most sensitive to triggering by stalk stimulation early in the NP cycle (Fig. 15), while it is most sensitive to triggering by single HP's late in the NP cycle (Fig. 14). This indicates that different mechanisms are involved in NP triggering by HP's and by stalk stimulation. It could by hypothesized, for example, that HP's, which are most effective just before the NP pacemaker is due to fire, act on the pacemaker itself while activity in the TS affects the pulse-producing mechanism more directly. The two sensitive periods for NP triggering, one coming early and the other late in the NP cycle, help to explain some features of the spontaneous NP activity. The HP triggering pattern indicates that at least some part of the NP system becomes increasingly excitable during the period between NP's, the time-course of this increasing excitability being measured in seconds. An increasing readiness to fire, developing slowly after firing, could lead to a pattern of single pulses separated by long intervals, the pattern in which NP's usually appear. On the other hand a peak

in the excitability of the NP system shortly after NP firing, as exemplified by the triggering sensitivity to stalk stimulation, could lead to repetitive firing at a high frequency, as is seen in NP bursts. In fact, finding that the NP system is quite excitable shortly after it has fired changes the question from 'why does the NP system fire in bursts?' to 'what terminates the bursts and why does the NP system not always fire in bursts?' Unfortunately answers cannot be given at this time.

	A	B	C	D
Mean (sec.)	16·1	17·4	16·0	15·7
S.D.	8·1	7·0	6·9	6·5

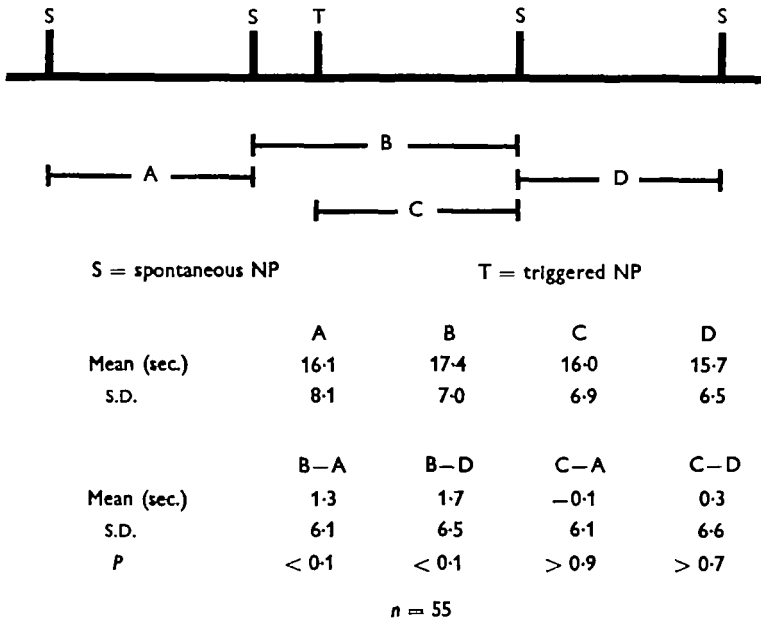|	B−A	B−D	C−A	C−D
Mean (sec.)	1·3	1·7	−0·1	0·3
S.D.	6·1	6·5	6·1	6·6
P	< 0·1	< 0·1	> 0·9	> 0·7

$n = 55$

Fig. 16. The intervals between spontaneous NP's and triggered NP's which were measured to answer the question 'does NP triggering by a stalk stimulus reset the NP pacemaker?' P is the probability that the average difference indicated or one greater would occur if the true difference were o (Student's t test, two-tailed).

Is the NP pacemaker reset when the NP system is triggered by a stalk stimulus? To answer this question, the records referred to above were re-examined to find sequences containing, in order, two spontaneous NP's, a triggered NP, and two more spontaneous NP's (Fig. 16). All of the spontaneous NP's had to be single pulses, i.e. they had to be separated from preceding and following spontaneous NP's by at least 1 sec. The following measurements were made on these sequences: the interval between the first two spontaneous NP's (A), the interval between the second and third spontaneous NP's (B), the interval between the triggered NP and the third spontaneous NP (C), and the interval between the last two spontaneous NP's (D). The differences B−A, B−D, C−A and C−D were also calculated. All sequences in which the interval between the triggered NP and the preceding spontaneous NP was greater than 3 sec. were discarded. Most of the triggering occurs in the first 3 sec. after an NP (Fig. 15), and eliminating those cases where there is apparently triggering at a longer interval minimizes the possibility that this apparent triggering is, in fact, just chance coincidence of a stimulus and a spontaneous NP. A total of fifty-five usable sequences was found in these records.

If the NP pacemaker were not reset during NP triggering, then interval B should be, on the average, equal to A or D or intermediate between A and D, and C should be

shorter than A or D. The two intervals between spontaneous NP's, A and D, were both measured because often the frequency of NP's slowly increases between bursts (Josephson, 1962). If the pacemaker were reset, then C should be equal to or inter-mediate between A and D, and B should be longer than A or D. The results of this analysis are given in Fig. 16. All the intervals are somewhat longer than usual, probably due to inhibition by the stalk stimulation (see below). Interval C is not significantly different from A or D, while B is apparently greater than A or D. This indicates that the NP pacemaker is reset when the NP system is triggered by stalk stimuli.

VI. *Interaction between polyps of a colony*

Often polyps can be found in a cluster of *Tubularia* which appear to branch from a common stalk. In some cases there is a partition of perisarc across the junction and hence no continuity of coenosarc tissue. Such branching has presumably arisen through the settling and subsequent development of an actinula larva upon an existent stalk. In other cases, however, there is coenosarc continuity at the junction, the gastro-vascular canals of the two polyps are continuous, and there is often continuity of the stalk-conducting systems across the junction (Josephson, 1965). It is not known whether this continuity is due to fusion of two individuals or branching during the growth of a single stalk.

Pacemaker interaction

Simultaneous recordings from the neck regions of two polyps with tissue continuity were made with six colonies (the word colony will be used here to refer to a collection of polyps with tissue continuity). Five of these colonies consisted of just two hydranths, one on each arm of a Y-shaped stalk. The sixth colony had four polyps. In each case there was frequent cross-talk between the NP systems of connected polyps. This is most clearly seen for NP bursts. The bursts in the two polyps on a Y-stalk almost always occurred nearly simultaneously (Fig. 17a); only very infrequently was there a burst in one polyp without there being a burst in the other. The bursts in all the polyps of the four-polyp colony appeared to be nearly coincident, as determined by recording from them two at a time. There is also interaction between single pulses (an NP in one polyp can trigger a pulse in the other) but most of the NP's recorded from a polyp of a colony arise within that polyp and are not externally triggered. The single NP patterns in the two polyps of a Y-stalk are usually similar but lack the striking correspondence seen in the bursts.

It appears that communication between the NP systems of polyps on a colony, at least for single pulses and some of the bursts, is via the stalk-conducting system termed 'the triggering system'. Of the three conducting systems in the stalk, only the TS is known to be capable of activating the NP system. The pattern of single-pulse triggering by other polyps is the same as that found when the TS is activated by stalk stimulation. Firing of an NP in one polyp is most likely to trigger an NP in a second, connected polyp if it comes shortly after the second polyp has already fired an NP. Triggered NP's in a polyp on a Y-colony are typically the second NP of a distinct pair, and follow NP's in the connected polyp by a reasonably constant interval (Figs. 17a, 18a). Further, the triggering latency for single-pulse triggering is about what should be expected from the conduction velocity of the TS and the distance between hydranths.

Table 1 gives some measurements of this latency from the five Y-colonies. For each colony ten latency measurements were made, five with the right polyp triggering the left and five with the triggering in the opposite direction. The usual recording speed allowed latency measurements to about 20 msec. The apparent conduction velocity between hydranths averages 14 cm./sec. From stalk-stimulation experiments it was
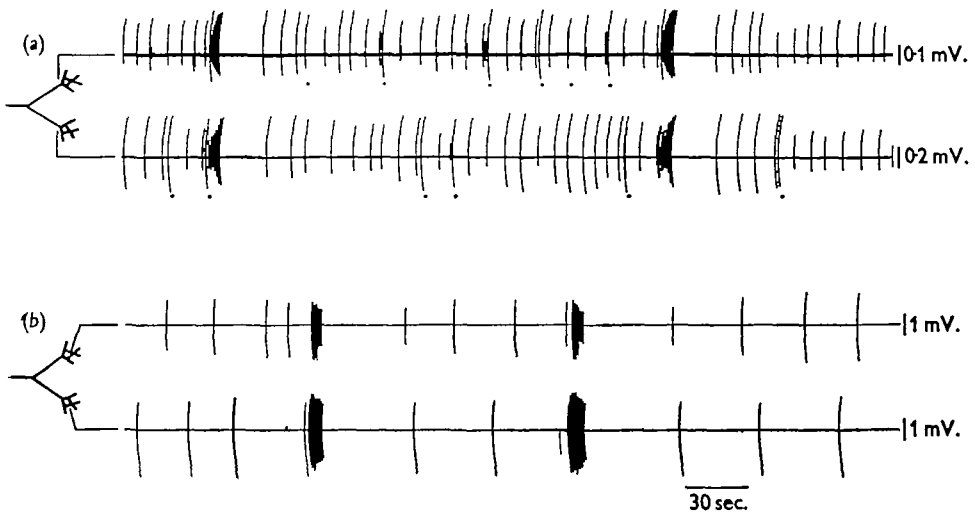

Fig. 17. NP's and HP's in connected hydranths, recorded with metal electrodes. The distance between the two hydranths was 56·2 mm. (*a*) Electrodes in polyp necks recording NP's. The dots indicate NP's which were probably triggered by activity in the other polyp. These NP's are the second of a pair and follow an NP in the opposite polyp by a reasonably constant interval. (*b*) Electrodes in polyp proboscides recording HP's.

Table 1. *The latency between spontaneous NP's in one polyp and triggered*
NP's in a connected polyp during interpolyp triggering in Y-colonies

Colony number	Average NP latency between polyps (msec., $N = 10$)	Distance between electrodes in polyp necks (mm.)	Apparent conduction velocity (cm./sec.)
1	180	25·7	14
2	250	36·8	15
3	370	55·2	15
4	290	32·0	11
5	170	21·9	13

concluded that the average interval between the arrival of TS activity at an electrode in the neck and the appearance of an NP at that electrode is 15 msec. (Josephson, 1965). Subtracting this time from the measured latencies in the present case raises the average conduction velocity to 15 cm./sec., which is slightly lower than, but close to, the 17 cm./sec. determined for the TS from stalk stimulation. The conduction velocity of the DOS is quite close to that of the TS, but it is unlikely that the DOS is the conducting system involved in colonial co-ordination because DOS pulses were not recorded when there was inter-polyp triggering, and distal tentacle opening, which is a consequence of DOS activity, was not seen. The above observations suggest that the TS is activated each time there is a spontaneous NP in a polyp and this TS activity can trigger NP's in any connected polyps which are in sensitive portions of their own

spontaneous cycles. It is unlikely that the TS is activated following triggered NP's, for this would lead to repetitive firing; each polyp on a Y-colony, for example, would be triggered and would in turn trigger the other polyp. Such cycling has not been seen.

There appear to be two mechanisms involved in the co-ordination of NP bursts between polyps of a colony. Sometimes the bursts appear to be co-ordinated by triggering through the TS, as the pulses in one hydranth follow those in the other by the same delay seen in single-pulse triggering (Fig. 18b). In other cases, however, the pulse delay between polyps is very short—less than 60 msec. in the Y-colonies investigated (Fig. 18c, e). This delay is far shorter than can be accounted for on the basis of TS conduction between the polyps. These two types of burst co-ordination will be termed long-delay co-ordination and short-delay co-ordination. Two of the five Y-colonies showed only long-delay co-ordination, one showed only short-delay co-ordination, and in two colonies both bursts with long-delay and bursts with short-delay co-ordination were seen in about equal numbers.

The NP frequency during bursts with long-delay co-ordination is characteristically lower than that during bursts with short-delay co-ordination. The average interval between pulses of the long-delay bursts ranged from 400 to 980 msec. In the short-delay bursts the average inter-pulse interval was usually between 90 and 280 msec., although during a portion of the record from one preparation it increased to 200–600 msec. One preparation produced a few bursts in which both long-delay and short-delay co-ordination were seen. The first pulses of these bursts were at high frequency and separated in the two hydranths by less than 40 msec., while the later pulses were at a distinctly lower frequency and separated in the two hydranths by over 150 msec. (Fig. 18d). There is frequently a change of leadership during the long-delay bursts, the polyp which is the follower during the first pulses of a burst becoming the leader for later pulses (Fig. 18b). There is no leadership change during the short-delay bursts, one hydranth leads and is the consistent leader from burst to burst. In two of the colonies which gave short-delay bursts the number of NP's during the bursts was almost always identical in the two connected hydranths. Most of the short-delay bursts recorded from these colonies (20 out of 24) began following one or more occurrences of single-pulse triggering. In the third colony with short-delay bursts either polyp could fire several pulses before the other began to respond, and only during the later portions of the bursts were the NP's in each hydranth in one-to-one correspondence. It was the polyp which led during the nearly synchronous portions of the bursts which most often started late in the burst and so produced fewer pulses (Fig. 18e).

The mechanism for co-ordinating the short-delay bursts is puzzling. Such co-ordination might be mediated by a fourth conducting system in the stalk, one which conducts at least four times faster than does the TS. The short-delay cannot be explained as a result of pronounced facilitation of the TS conduction velocity, for if facilitation of conduction velocity were involved one would expect the first triggered pulse in a polyp to have a normal delay and only succeeding pulses to have shorter delay, whereas the short-delay bursts begin with short delay. The explanation which seems most likely is that there are pacemakers in the stalks of Y-colonies other than those in the polyp neck regions. A pacemaker near the junction or in the common portion of the stalk below the junction could drive the two polyps nearly synchronously through the TS, for the conduction distances would be nearly the same. If it is supposed

that such pacemakers produce bursts with a higher pulse frequency than the neck pace-
makers, the frequency difference between long-delay and short-delay bursts could be
explained. The end of the common stalk which was cut to isolate the Y-preparation
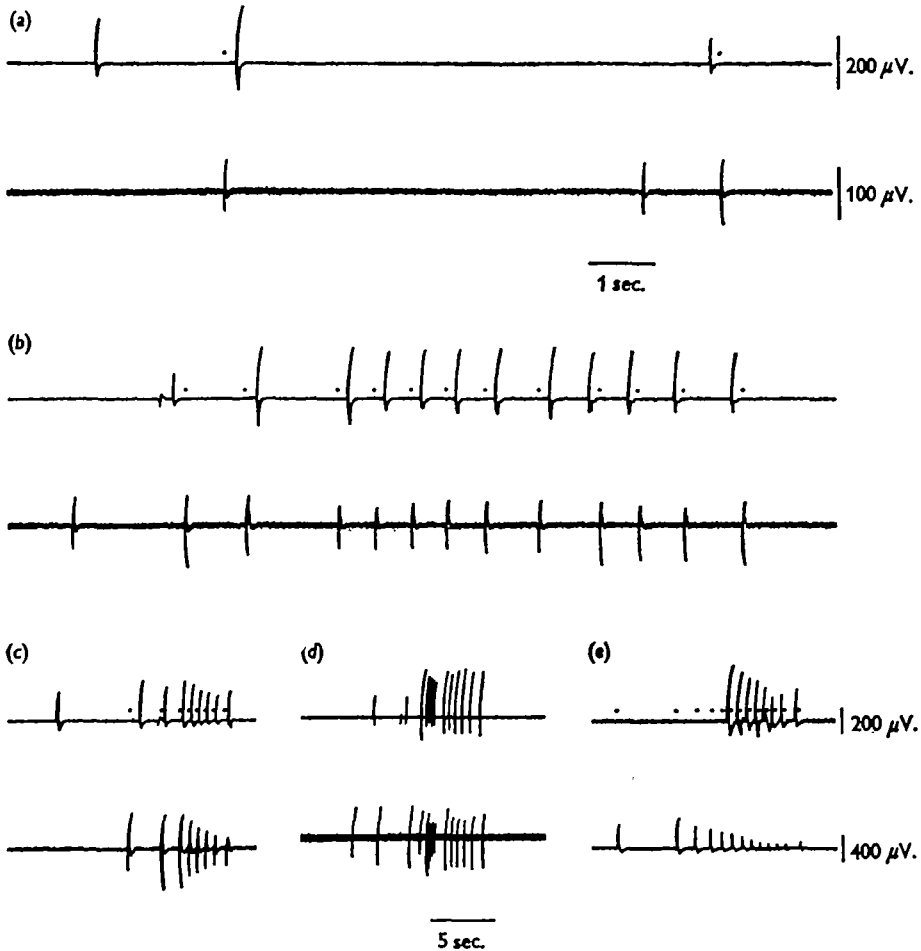seems a likely site for such adventitious pacemakers, but this has yet to be investigated.

Fig. 18. Triggered NP's and NP bursts in connected polyps of Y-colonies. The recording was
as shown in Fig. 17a, the upper trace in each case coming from the neck of one polyp and the
lower trace from the neck of the other polyp. (a)–(d) were recorded with metal electrodes from
a colony in which the hydranths were 21·9 mm. apart, (e) was recorded with suction electrodes
from another colony in which the hydranths were 32 mm. apart. Dots near the upper traces
mark the onset of pulses in the lower traces. The amplitude scales in (a) apply to records (a)–(d)
the time-scale in (a) applies to (a)–(c) and (e). (a) Single-pulse triggering, first the lower-trace
polyp triggering the upper and then triggering in the reverse direction. (b) A long-delay burst.
The first potential in the upper trace is an HP. This is followed by an NP which triggers an
NP in the lower-trace polyp. Note the change in leadership during the burst; the lower-trace
polyp being the leader for seven pulses and the follower for the remaining four. (c) A short-
delay burst which begins following an example of single-pulse triggering. Note the small
latency between channels and the lack of leadership change. (d) An example of a mixed burst.
The first burst pulses are at high frequency and the short-delay type, the later pulses are at
lower frequency and of the long-delay type. (e) A short-delay burst from another colony.
The upper-trace polyp began to fire late in the burst, but leads the lower-trace polyp during the
part of the burst in which they are both active. (Retouched for reproduction.)

The relation between HP's in connected hydranths is as would be predicted from the loose coupling between NP systems of connected polyps and the loose coupling between NP and HP systems in a single polyp. Single HP's or short HP bursts are not co-ordinated between connected polyps, while the longer HP bursts, those that occur in conjunction with NP bursts, are nearly coincident throughout a colony (Fig. 17b).

Behavioural consequences

Because of the indirect coupling of HP systems of connected polyps the concert activity of polyps in a colony is to some degree co-ordinated. The less-vigorous concerts (those that occur with independent firing of the HP system) are generally not correlated between connected polyps, while the vigorous concerts (those that occur with NP–HP bursts) are generally coincident in connected polyps. Neck contraction occurs during the longer NP–HP bursts, and concerts with neck contraction are usually synchronous throughout a colony. Such synchronization is of obvious advantage if it is assumed that the function of concerts with neck contraction is to transfer fluid and partially digested food between the two enteron chambers of a polyp. Were neck contractions not co-ordinated, sometimes one polyp would contract its neck while a connected polyp would be relaxing following such contraction. In this case neck contraction would force fluid down the stalk toward the relaxing polyp rather than distally into its own oral chamber; there would be no tidal exchange between the two enteron chambers of the contracting polyp. As it is, neck contractions are synchronized throughout a colony, the pressure in the connected neck and stalk compartments must increase throughout the colony during neck contraction, and the passage of fluid into the oral chambers of hydranths throughout the colony is facilitated.

Fulton (1963) found that peristaltic waves in polyps of the hydroid *Cordylophora* are synchronized throughout a colony. *Tubularia* differs from *Cordylophora* in that not all peristaltic waves are synchronized, and in the more rapid time-scale of events in *Tubularia*. Peristaltic waves appear 2–6 times per hour in *Cordylophora* and require time-lapse photography to be appreciated. Peristaltic waves appear up to several times a minute in *Tubularia* and are rapid enough to be easily seen in 'real' time. The great difference in the time-scale of events may indicate that different mechanisms are involved in synchronization in the two genera.

VII. *Inhibition of spontaneous activity by stalk stimulation*

Although stalk stimulation can trigger NP's in a polyp, prolonged stimulation at low frequency usually reduces the frequency of both electrical potentials from polyp pacemaker systems and spontaneous tentacle movements. Some inhibition of spontaneous activity is often seen with stimuli 20 sec. apart, and the inhibition generally becomes quite pronounced with stimuli 5 or 2·5 sec. apart. The intensity as well as the frequency of tentacle movements is affected by low-frequency stimulation; proximal tentacle flexions become quite slow and the whole polyp appears lethargic, with the exception of the distal tentacles which continue to flare outward with each shock. Inhibition sometimes does not begin immediately with stimulation; the frequency of polyp activities may slowly decline and take 20–40 sec. to reach a stable level. In some cases, especially at higher stimulus frequencies (e.g. one shock each

2·5 sec.), the inhibition is temporary and after several minutes the polyp regains its original activity level or sometimes becomes hyperactive. Some polyps become hyperactive at the termination of a 5 min. stimulus period, but others show a slow return to the original activity level.

Figs. 19, 20 and Table 2 illustrate some of the inhibitory effects of low-frequency stalk stimulation. Fig. 20 gives the results of a series of experiments testing the effect of stimulus frequency. In these experiments NP's were recorded from the neck, and polyp concerts were counted for a 5 min. period. This was followed by a 5 min. period during which the DOS threshold was determined three times and an average value computed. The stalk was then stimulated at one shock per 20 sec. for 5 min., while concerts and NP's were again recorded. The stimulus intensity was twice DOS threshold. A 5 min.
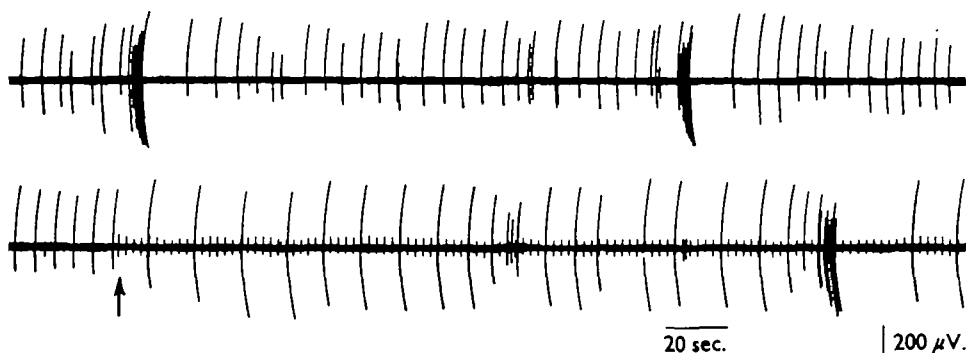

Fig. 19. Inhibition of NP's during stalk stimulation at one shock per 2·5 sec. The NP's were recorded with a metal electrode in the polyp neck. The arrow marks the onset of stimulation; the small potentials thereafter are shock artifacts. There are nine NP's in the 2 min. period following the beginning of stimulation as compared with twenty in the 2 min. period immediately preceding stimulation.

rest period was allowed following the stimulation, after which the whole sequence was repeated with stimuli at one per 10 sec., one per 5 sec., and then one per 2·5 sec. This was repeated with ten different polyps. In the analysis of these records, a group of three or more NP's with an interval of 1 sec. or less between successive NP's was counted as an NP burst. 'Non-burst NP's' refers to NP's other than those coming in bursts. All NP's, whether spontaneous or triggered by the stimulation, were counted. The number of concerts recorded declined with increasing stimulus frequency over the whole range tested. The number of NP's and NP bursts declined with increasing stimulus frequency to one shock per 5 sec. At one shock per 2·5 sec., however, the average number of NP's and NP bursts was somewhat greater than it was with stimuli at one per 5 sec. and the variation from polyp to polyp, as shown by the standard deviations, increased greatly for all NP parameters. At this frequency some of the polyps were strongly inhibited but others became hyperactive toward the end of the stimulation period. The decline in the total number of NP's with stimuli at 20 and 10 sec. intervals was due to a decrease in the number of NP bursts, as the frequency of non-burst NP's did not appreciably change until higher stimulus frequencies were used.

Table 2 is based on the same experiments as Fig. 20, and some similar experiments during which HP's were recorded from the polyp proboscis. The observation periods preceding and during stimulation at one per 5 sec. were chosen because at this
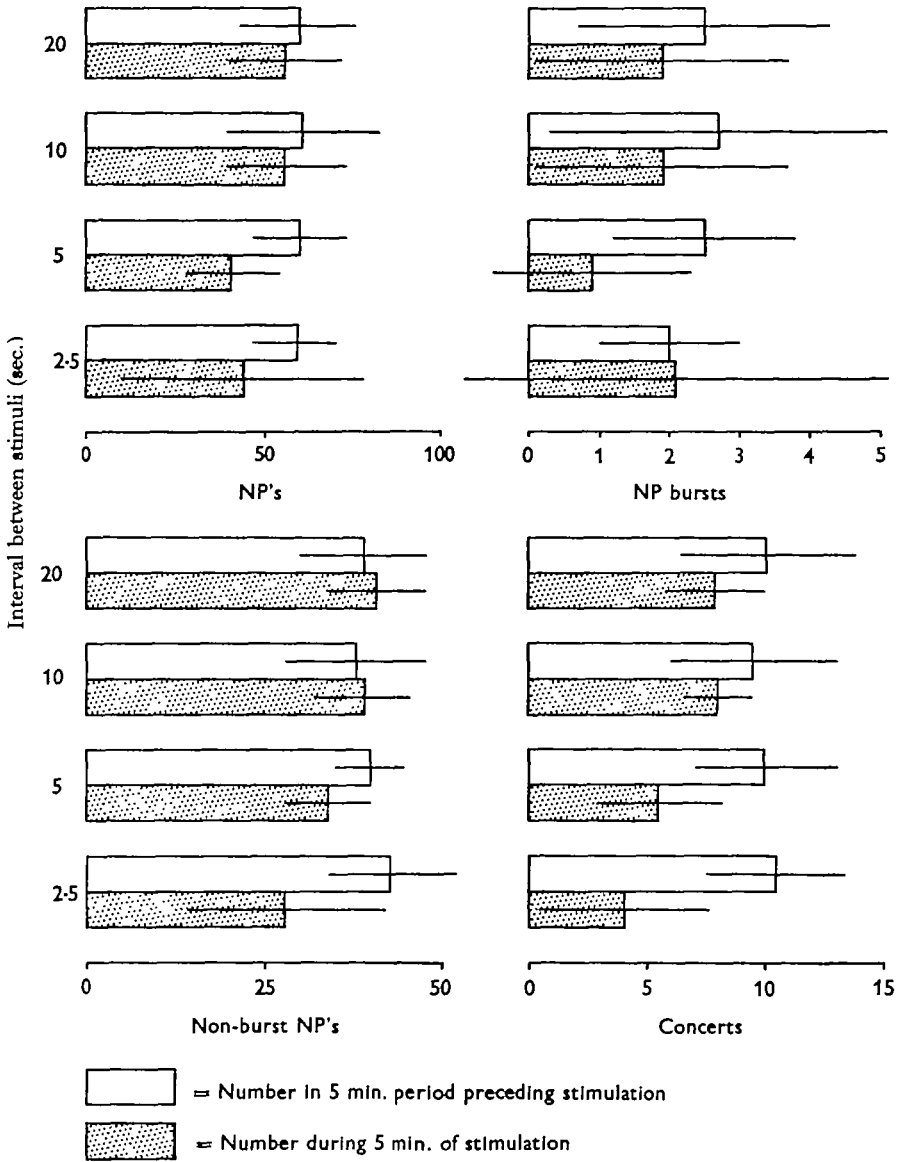
Fig. 20. The number of events during a 5 min. period of stimulation compared with the number in a preceding 5 min. period without stimulation. The bars are the means of ten determinations; the thin lines show standard deviations.

frequency inhibition is quite pronounced and yet only rarely does a polyp become hyperactive. The computed measure of inhibition, percentage inhibition, is defined as follows:

A = number of events in 5 min. period preceding stimulation;

B = number of events during 5 min. of stimulation;

$$\text{percentage inhibition} = 100\left(\frac{A-B}{A}\right).$$

It can be seen from Table 2 that there was significant inhibition of all the spontaneous activity parameters measured. A few experiments indicate that the frequency of TP's recorded from a tentacle is similarly reduced during low-frequency stalk stimulation, but these experiments are yet too fragmentary to be conclusive.

Inhibition of spontaneous activity in the distal stalk and hydranth during stimulation of the proximal stalk indicates that there is a conducting system in the stalk mediating this inhibition. Of the three known conducting systems in the stalk, the TS, DOS and SS, only the SS can be eliminated as being involved in this inhibition; stimuli which are well below the SS threshold can cause clear inhibition. The threshold for inhibition appears to be close to the thresholds of the DOS and TS, suggesting that it is one of these which mediates inhibition and not another, yet undiscovered, conducting system.

Table 2. *Inhibition of spontaneous activity in* Tubularia *polyps during stalk stimulation at one shock per 5 sec.*

(Percentage inhibition = percentage reduction in the number of events during a 5 min. stimulation period as compared with a preceding 5 min. period without stimulation. N = number of determinations, each with a different polyp. P = probability that the average value for percentage inhibition is not significantly different from zero.)

	Average inhibition (%)	N	S.D.	P
Total NP's	33	10	13	< 0·01
NP bursts	70	9*	45	< 0·01
Non-burst NP's	15	10	12	< 0·01
Concerts	40	10	31	< 0·01
HP's	51	5†	34	< 0·05

* One trial in which there were no NP bursts in either the prestimulation period or the stimulation period has been omitted.

† One trial in which the polyp became hyperactive and went into a continued spasm during stimulation has been omitted.

The obvious experiment, the use of stimuli whose intensity is above the TS threshold but below the DOS threshold, has been tried a few times but not yet successfully. The DOS and TS thresholds are quite close and somewhat variable; in the few attempts made it has not been possible to stimulate for any time without frequently either activating the DOS or failing to activate the TS. Either the TS or the DOS is probably involved in the inhibition, but which one remains to be determined.

Inhibition of spontaneous activity by external stimulation is known for other coelenterates, although in no case is the mechanism of inhibition understood. In a number of hydromedusae, stimuli which cause contraction of the radial musculature of the bell also inhibit spontaneous beating of the bell (Horridge, 1955). Similar inhibition of spontaneous beating by external stimulation has also been found in some scyphomedusae (Horridge, 1956a, b). Prolonged low-frequency electrical stimulation (one shock each 10 sec.) can reduce the frequency of spontaneous column contractions in the anemone *Metridium* (Batham & Pantin, 1950). The responses of isolated column rings from the anemone *Calliactis* to electrical stimulation have been interpreted as being due to both excitatory and inhibitory effects of the stimulation (Ewer, 1960). One of the pacemaker systems of *Hydra*, the contraction-burst system, usually produces a burst of pulses every 5–10 min. (Passano & McCullough, 1964).

The pulses of these bursts are associated with column shortening. Activation of the contraction-burst system every 15–30 sec. with single shocks, each of which evokes a single contraction-burst pulse, generally blocks the occurrence of normal bursts. Further, sudden illumination can inhibit a contraction burst in progress. The frequency of spontaneous column contractions in *Hydra* is reduced when the animals are feeding on *Artemia* or when reduced glutathione is added to the surrounding water (Rushworth *et al.* 1964). The chondrophore *Porpita* shows spontaneous, concerted oral flexions of its tentacles very similar to the concerts of *Tubularia* (Mackie, 1959). A sharp mechanical stimulus to *Porpita* evokes aboral bending of the tentacles. If the stimulus is given during a concert, the concert is interrupted and is not resumed after the aboral response. A possible example of inhibition which may be quite similar to that in *Tubularia* is found in the hydroid *Corymorpha*. Parker (1919) describes a feeding response in *Corymorpha* which begins with a concerted elevation of the proximal tentacles about the proboscis and continues with column bending which brings the distal tentacles to the mud surrounding the polyp. This response is repeated rhythmically at intervals of about 3 min. when the polyp is in quiet water; it is not seen when the polyp is in flowing water. It seems likely that flowing water mechanically stimulates the polyp stalk or hydranth, this stimulation inhibiting the feeding response.

<div align="center">DISCUSSION</div>

The organization of pacemaker and conducting systems in Tubularia

The spontaneous behaviour of *Tubularia* results from the interaction of a number of pacemaker systems, there being a major pacemaker system in the neck region, another in the hydranth, and additional pacemakers in the tentacles. Individual pacemakers must be postulated for each proximal tentacle. Each tentacle has its own pattern of spontaneous oral flexions and the electrical activity recorded from two proximal tentacles is asynchronous except during HP bursts. Single distal tentacles or small groups of tentacles often show spontaneous movements in either the oral or aboral direction. This is taken to indicate that there are pacemakers controlling both the oral and aboral musculature of the distal tentacles, although electrical correlates of their activity have not yet been recorded.

There can be no doubt that the activity in the NP and HP systems is initiated by endogenous pacemakers and is not the result of chain reflex mechanisms involving stimulation of mechanoreceptors by the animal's movements. HP's and NP's continue to appear long after movements of the animal have ceased during $MgCl_2$ anaesthetization. In an earlier discussion of the chondrophore *Porpita*, which shows 'concerts' very similar to those of *Tubularia*, Mackie (1959) proposed a co-ordinating and impulse-generating system of nerves which were supposed to be activated periodically by information from stretch receptors in the tentacles. In the light of the work with *Tubularia* it seems more probable that *Porpita* has pacemakers capable of producing the characteristic concert pattern without help from feedback systems. There are two principal pacemaker systems in *Hydra* (see below). Passano & McCullough (1964, 1965) have clearly shown that there are many potential pacemakers in each of these systems, and the initiation of activity frequently shifts from one pacemaker locus to another. Similarly there are probably many potential pacemakers in the HP and NP systems of

Tubularia. The results of experiments with sectioning of the upper stalk indicate that there are a number of potential pacemakers in the NP system (Josephson, 1962), and the changing latencies between NP's recorded at two points on the neck suggest that the pacemaker locus is not fixed but can change from pulse to pulse.

Although a *Tubularia* polyp contains a number of pacemakers, it is not an anarchy. Periodically, during most NP bursts, the individual pacemaker systems are driven to fire in concurrence. The NP system can drive the HP system and, probably through the HP system, the tentacle systems as well. The behaviour of *Tubularia*, like that of *Hydra* (Passano & McCullough, 1963), is controlled by pacemakers in a hierarchical
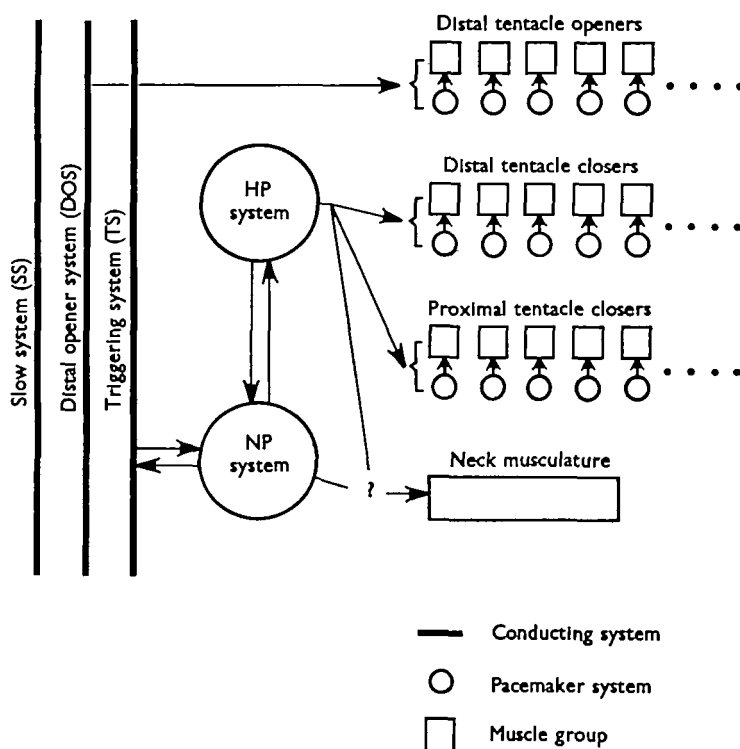

Fig. 21. Some of the interactions between conducting systems, pacemaker systems and effectors found in *Tubularia*.

arrangement. But the interaction between pacemakers is not just unilateral, for firing of the HP system can trigger NP's. Fig. 21 is a diagrammatic representation of some of the interactions between pacemaker systems, conducting systems and effectors described in this and a previous paper (Josephson, 1965). The control of the neck musclature is problematical. Neck contraction of the sort leading to tidal exchange occurs during the longer NP bursts when both the NP and HP systems are active, but precisely how the neck contraction is initiated is uncertain. This uncertainty is represented by the question-mark in Fig. 21. The mechanisms controlling the slow contraction of the neck muscles which lead to swaying of the hydranth on the stalk are completely unknown and so are not included in the diagram. Other elements involved

in the behaviour of *Tubularia* have been omitted from Fig. 21 because of gross uncertainties about their nature and mode of action. It is not known what initiates the peristaltic contraction of the circular muscles of the proboscis, nor how this peristaltic contraction is propagated down the proboscis. Similar uncertainty about the conducting system mediating the polyp inhibition seen during stalk stimulation and uncertainties about which pacemakers are directly affected has caused this inhibitory control to be omitted from the diagram. There is some indication that there is direct interaction between the NP system and the pacemakers controlling flexion of individual proximal tentacles, but this remains to be verified. Future investigations will undoubtedly reveal still other inadequacies. The complete control diagram can be expected to be far more elaborate than the one given.

The mechanisms controlling behaviour in *Tubularia* are quite complex, far more complex than we, at least, had expected before this study on *Tubularia* and the similar work by Passano & McCullough with *Hydra* were begun. Many fundamental problems remain to be solved before an adequate description of the complete control of behaviour in *Tubularia* can be formulated. The interactions between pacemakers must not only be identified but also quantified, perhaps most usefully as probability functions. The influence of such factors as age and nutritional state must be better determined. The cellular elements of the pacemaker systems and conducting systems remain to be identified. There is some evidence that the DOS is nervous (Josephson, 1965), and that the potentials associated with the NP and HP systems are due to activity of the external epithelial layer (see below), but more information is needed. The pacemakers of the NP and HP systems may be parts of the potential-producing cells themselves, or, as Passano & McCullough suggest, different sorts of cells which trigger the potential-producing cells to fire. The identification of the elements involved in pacemaker and conduction activity is likely to be difficult, and will probably require newer and more imaginative techniques than have yet been applied to coelenterates. But the task is certainly worth while, for we are probably closer to an understanding of the mechanisms controlling behaviour in *Tubularia* or *Hydra* than in any other multicellular animals.

One of the features long thought to be characteristic of the behaviour of coelenterates is a relative independence of different parts of the organisms (e.g. Parker, 1919; Hyman, 1940, p. 399; Nicol, 1960, p. 424; Bullock & Horridge, 1965). This concept is based to a large extent on the fact that isolated parts of a coelenterate usually perform much the same as they did when attached. Our results have emphasized rather the interdependence of parts and the co-ordination of their activities. *Tubularia* now appears to be somewhat like a symphony orchestra; any member, when isolated, can perform solo; but when assembled the activities of the members show a high degree of co-ordination.

The origin of pacemaker system potentials

What is the source of the spontaneous electrical pulses in *Tubularia*? Are they the result of nervous activity as was earlier suggested (Josephson, 1962)? At the present time two lines of evidence suggest that they are not.

The first type of evidence is of an exclusive nature; the nervous system appears to play a distinctly different role. Leghissa (1950), who used silver impregnation methods

with *Tubularia*, reports that there is a single nerve plexus in *Tubularia* hydranths. Selective neurone staining with methylene blue also indicates that there is but a single nerve plexus in hydranths of *Tubularia* (Mackie, unpublished observations). A single nerve plexus should function as a single conducting system if it operates as nerve nets are thought to do in other coelenterates (Josephson, 1964). Evidence has been presented elsewhere (Josephson, 1965) that the conducting system in the stalk of *Tubularia* which courses through the hydranth and causes opening of the distal tentacles is nervous, and is probably the nerve net of the hydranth. The potentials associated with activity in this conducting system are considerably smaller and shorter than the spontaneous pulses. Hence the spontaneous pulses must originate in a different and presumably non-nervous system.

Secondly, the large size of the spontaneous potentials would appear to militate against their being nervous. Potentials recorded with suction electrodes on the collar or proboscis are consistently larger than 100 μV. and are sometimes as large as 5 mV. The neurones of the net are less than 5μ in diameter and are quite dispersed. The orifice of the suction electrodes used is many times larger than the diameter of the neurones, and even with the most favourable placement there must be much more non-nervous tissue beneath the electrodes than nervous tissue. Under these conditions it seems quite unlikely that firing of the fine neurones of the net could give rise to potentials of the size recorded.

A possible clue to the source of the spontaneous potentials is their usual positivity in the distal hydranth when recorded with suction electrodes, and especially the reversal of NP's from negative to positive pulses when a suction electrode is moved from the neck to the adjacent collar (Fig. 9). A suction electrode can operate in two ways. If it is near but not touching an active area it will act as an ordinary external electrode. A travelling wave of surface negativity will be seen as a predominantly negative potential. If the suction electrode is on an active area it can inactivate it, either by injury or by preventing current flow through the tissue enclosed within the electrode orifice. In this case a travelling wave of surface negativity will be seen as a monophasic positive potential as the inactivated area serves as a current source for the surrounding active tissue (e.g. Hoffman, Cranefield, Lepeschkin, Surawicz & Herrlich, 1959). A suction electrode on the neck of *Tubularia* is separated from the epithelium by a layer of perisarc, while it rests directly on the epithelium when on the collar. When the electrode is on the perisarc it records negative NP's; immediately adjacent on the epithelium it usually records positive NP's. This suggests that it is the epithelial surface itself which is the source of the NP's, and further, that the NP's result from the epithelial surface becoming transiently negative with respect to the surface of surrounding inactive tissue. There is some indication that the pulse form with suction electrodes can be altered by changing the magnitude of the suction. In the present study the suction was kept minimal to avoid suction potentials. The complex and partially negative potentials frequently seen from the collar or proboscis may have been a result of at least partial invasion of the tissue beneath the electrode because of the electrode suction being insufficient to inactivate the underlying tissue completely. Although a transient negativity of the epithelial surface would explain the polarity of the potentials recorded, there are other possible explanations. For example, the potential reversal when an electrode is moved from neck to collar may result from

distortion of underlying tissue when the electrode is on the compliant epithelium, this distortion being prevented when the electrode is on the more rigid perisarc.

We would like to suggest the following hypothesis as to the source of the spontaneous pulses in *Tubularia*. Portions of the surface epithelium are organized into conducting systems. Conduction in these systems is manifest by a travelling wave of surface negativity. The epithelium of the proboscis forms part of what has been termed the HP system, the neck epithelium forms the NP system. Conduction in these systems must be supposed to account for (*a*) the unitary nature of the responses in the neck and hypostome, (*b*) the rather long latencies often seen between the onset of pulses at two points in the neck, and (*c*) the fact that HP's in the proboscis are everywhere about equally large, which would not be the case if the potentials originated from a single, localized source. Conduction between epithelial cells might be a result of chemical transmitter mechanisms, but more likely the mechanism is current flow in local circuits as appears to be the case for heart muscle and visceral smooth muscle in vertebrates (Woodbury & Crill, 1961; Barr, 1963; Prosser, 1962). Intrusions of one ectodermal epithelio-muscular cell into another and desmosome-like connexions between the muscular bases of these cells have been found in *Hydra* (Hess, 1961). Such contact may provide the basis for cell-to-cell transmission. Whether transmission between cells is chemical or electrical, there should be conduction barriers between the different systems such that one system can fire without activating the others. These barriers might be bands of high resistance between epithelial cells otherwise joined by low-resistance pathways. The small, positive HP's in the neck and NP's in the proboscis are probably a result of passive current flow through inactive regions. The barriers between conducting systems should not be permanent, but rather should be capable of being surmounted as when the NP system fires in a burst and drives the HP system. We are suggesting that there can be facilitatory processes between epithelial areas analogous to the interneural facilitation between areas of the nerve nets of some coelenterates (e.g. Pantin, 1935).

The idea of non-nervous conduction in coelenterates is certainly not new. For example, Parker (1919) reported that a ring of contraction develops around the column of the hydroid *Corymorpha* when it is stimulated at one point. This response is not abolished by chloretone anaesthetization, which Parker thought abolished nervously controlled responses. Several cases are known in coelenterates where conduction through a tissue can be demonstrated but where nerve cells have yet to be found (Mackie, 1964, 1965*a*). It has been suggested that the large, spontaneous potentials of *Hydra*, which are quite similar to those of *Tubularia*, may be a result of intermuscular rather than neuronal transmission (McCullough, 1963). Pantin (1956) has pointed out that in the early evolution of metazoa a nervous system was probably added to more primitive co-ordinating mechanisms, the resulting combination having the potentiality of more complex activity and response. It appears that in at least some coelenterates a proportionally greater part of the organismal behaviour is controlled through primitive, non-nervous mechanisms than is the case in the higher animals which have been investigated.

Tubularia *and* Hydra

In a recent series of papers, Passano & McCullough have described many features of the behaviour and electrical activity of several *Hydra* species (McCullough, 1962,

1963; Passano, 1962; Passano & McCullough, 1962, 1963, 1964, 1965). *Hydra* and *Tubularia* are structually somewhat disimilar and have quite different behavioural capabilities. *Tubularia* is sessile and, because of endodermal supporting elements, has a rather fixed shape, while *Hydra* is capable of great elongation and contraction and can move about in several ways. Nevertheless there are a number of similarities in the mechanisms controlling behaviour in the two genera. Both have several through-conducting systems in the polyp and in both there are multiple pacemakers whose activities determine the spontaneous behaviour of the polyp.

There are two principal pacemaker systems in *Hydra*: the contraction-burst system (CB system) and the rhythmic potential system (RP system). The contraction-burst system occasionally produces single pulses or short bursts of 2–4 pulses, but most often it fires in prolonged bursts of 5–15 pulses, the number of pulses in the longer bursts depending on the species used. The pulses are conducted in the column at 15 cm./sec. Each of the CB pulses is followed by shortening of the column and tentacles. Extirpation experiments, two-channel recording, and the effects of localized illumination indicate that the CB pacemakers are in the sub-hypostome region of the polyp. Thus the CB system of *Hydra* resembles the HP system of *Tubularia* in (1) its direct relation to polyp behaviour, (2) its firing pattern, single pulses and bursts of pulses, and (3) the location of its pacemakers in the distal polyp.

Activity in the rhythmical potential system usually appears as single pulses separated by 6–60 sec. These pulses, to use Passano & McCullough's terminology, are cryptic, they do not appear to be directly related to overt behaviour. Extirpation experiments, the effects of localized light stimulation, and two-channel recording all indicate that the pacemakers initiating these pulses are near the basal disk of the polyp, although potential pacemakers are found throughout the column. The pulses are conducted in the column at about 4 cm./sec. Occasionally pulses are recorded in *Hydra* which resemble the RP pulses in shape and conduction velocity, but differ in that they appear in bursts (pre-locomotor bursts = PLB's), up to one pulse per sec., rather than single pulses. These bursts occur when the tentacles of *Hydra* are attached to the substrate, just prior to the execution of a somersault step. The pacemakers initiating these bursts are in the sub-hypostome region rather than the polyp base as is the case for the single pulses. The similarity of the PLB pulses and single RP pulses in shape and conduction velocity suggests that they are conducted in the same system, although this is not yet certain. Thus the rhythmic potential system of *Hydra* and the NP system of *Tubularia* are similar in their indirect relation to polyp behaviour and in the usual basal location of their pacemakers. If the PLB pacemakers of *Hydra* are considered to be part of the RP system, then this system produces single pulses and bursts as does the NP system of *Tubularia*, although the pacemakers for the pulses and bursts in *Hydra* are in different locations, which does not appear to be the case for *Tubularia*. We suggest that the HP system of *Tubularia* is homologous with the contraction-burst system of *Hydra*, and the NP system of *Tubularia* homologous with the rhythmic potential system of *Hydra*. These homologies can only be suggestions until more is known about these systems in *Tubularia* and *Hydra*, and until something is known about similar control systems in other hydroids.

The coupling between the two principal pacemaker systems of *Hydra* is far looser than the coupling between the HP and NP systems of *Tubularia*. It has been suggested

that activity in the RP system of *Hydra* inhibits the CB system (Passano & McCullough, 1963; McCullough, 1965), but this inhibition is often not obvious in electrical recordings from *Hydra*. The possibility exists that the contraction-burst system and the rhythmic potential system fire nearly synchronously during contraction bursts, as the NP and HP systems of *Tubularia* fire nearly synchronously during bursts. Contraction-burst pulses are quite large (up to 30 mV.) and would effectively obscure the much smaller rhythmic potentials (up to 0·6 mV.). But the occasional occurrence of rhythmic potentials between successive potentials of a contraction burst (Passano & McCullough, 1962) suggest that the two pacemaker systems of *Hydra* are relatively independent even during bursts.

One interesting difference between *Tubularia* and *Hydra* is the relatively slow time-scale of events in *Hydra*. The pulses in *Hydra* are about five times longer than those in *Tubularia*, and the frequency of single pulses, the frequency of pulse bursts and the frequency of pulses within a burst are all about five times slower in *Hydra* than in *Tubularia*.

SUMMARY

1. The spontaneous behaviour of the hydroid *Tubularia* results from the activity of a number of pacemaker systems in the polyp, there being a major pacemaker system in the distal stalk (the NP system), another in the hydranth body (the HP system) and individual pacemakers in each of the tentacles. The NP system produces single electrical pulses and bursts of three or more pulses, the HP system itself produces single pulses and short bursts of 2–4 pulses, and the tentacle pacemakers fire irregularly.

2. The HP and NP systems are mutually interactive. When the NP system fires in a burst it usually drives the HP system to fire concurrently. Single firing by the NP system sometimes triggers the HP system to fire, and single HP firing can trigger the NP system. Single HP firing is most likely to trigger the NP system if it comes long after the last NP firing, i.e. late in the NP spontaneous cycle. When the HP system independently fires a burst or is driven by the NP system to fire a burst, it drives pacemaker systems in the proximal tentacles to fire concurrently.

3. Firing of the HP system is associated with a behavioural response termed a concert. During a concert the proximal and sometimes the distal tentacles are synchronously elevated. During the longer NP–HP bursts the neck contracts. A peristaltic wave sweeps down the proboscis following the tentacle elevation. Concerts without neck contraction result in mixing of fluid in the enteron chamber of the proboscis; concerts with neck contraction result in tidal exchange of fluid between the neck chamber and the proboscis chamber.

4. Stimulation of the conducting system in the stalk termed the triggering system (TS) can trigger the NP system to fire. A stalk stimulus is most likely to trigger the NP system if it comes within a few seconds of NP firing, i.e. early in the NP spontaneous cycle.

5. Firing of the NP system of one polyp in a colony can trigger the NP systems of connected polyps. Interpolyp triggering is probably mediated through the TS conducting system. The coupling between NP systems of connected polyps results in synchronization of NP bursts throughout a colony. Because of the coupling between NP and HP systems within single polyps the longer HP bursts, which are those

occurring when the HP system is driven by the NP system, are also usually synchronous in connected polyps. This results in concerts with neck contraction being nearly synchronous throughout a colony. Such synchronization facilitates transfer of fluid between the two enteron chambers of polyps during concerts.

6. Prolonged, low-frequency stalk stimulation inhibits spontaneous activity of a polyp, the inhibition increasing with increasing stimulus frequency.

7. The large size of the spontaneous electrical pulses suggests that they are not the result of nervous activity, but possibly due to activity of more primitive co-ordinating mechanisms.

REFERENCES

ALLMAN, G. J. (1871). *A Monograph of the Gymnoblastic or Tubularian Hydroids. I. The Hydroida in General.* Ray Society, London.

BARR, L. (1963). Propagation in vertebrate visceral smooth muscle. *J. Theoret. Biol.* **4**, 73–85.

BATHAM, E. J. & PANTIN, C. F. A. (1950). Phases of activity in the sea-anemone, *Metridium senile* (L.), and their relation to external stimuli. *J. Exp Biol.* **27**, 377–99.

BEUTLER, R. (1924). Experimentelle Untersuchungen über die Verdauung bei *Hydra. Z. vergl. Physiol.* **1**, 1–56.

BEUTLER, R. (1925). Beobachtungen an gefütterten Hydroidpolypen. *Z. vergl. Physiol.* **3**, 737–75.

BULLOCK, T. H. & HORRIDGE, G. A. (1965). *Structure and Function in the Nervous Systems of Invertebrates.* San Francisco: W. H. Freeman Co. (In the Press.)

EWER, D. W. (1960). Inhibition and rhythmic activity of the circular muscles of *Calliactis parasitica* (Couch). *J. Exp. Biol.* **37**, 812–31.

FULTON, C. (1963). Rhythmic movements in *Cordylophora. J. Cell. Comp. Physiol.* **61**, 39–52.

HESS, A. (1961). The fine structure of cells in *Hydra.* In *The Biology of Hydra and of Some Other Coelenterates,* ed. H. M. Lenhoff and W. F. Loomis, pp. 1–49. Coral Gables, Florida: Univ. of Miami Press.

HOFFMAN, B. F., CRANEFIELD, P. F., LEPESCHKIN, E., SURAWICZ, B. & HERRLICH, H. C. (1959). Comparison of cardiac monophasic action potentials recorded by intracellular and suction electrodes. *Amer. J. Physiol.* **196**, 1297–1301.

HORRIDGE, G. A. (1955). The nerves and muscles of medusae. IV. Inhibition in *Aequorea forskalea. J. Exp. Biol.* **32**, 642–8.

HORRIDGE, G. A. (1956a). The nervous system of the ephyra larva of *Aurellia aurita. Quart. J. Micr. Sci.* **97**, 59–74.

HORRIDGE, G. A. (1956b). The nerves and muscles of medusae. V. Double innervation in Scyphozoa. *J. Exp. Biol.* **33**, 366–83.

HYMAN, L. H. (1940). *The Invertebrates: Protozoa through Ctenophora.* New York: McGraw-Hill.

JOSEPHSON, R. K. (1961). Colonial responses of hydroid polyps. *J. Exp. Biol.* **38**, 559–77.

JOSEPHSON, R. K. (1962). Spontaneous electrical activity in a hydroid polyp. *Comp. Biochem. Physiol.* **5**, 45–58.

JOSEPHSON, R. K. (1964). Coelenterate conducting systems. In *Neural Theory and Modeling,* ed. R. F. Reiss, pp. 414–22. Stanford, California: Stanford University Press.

JOSEPHSON, R. K. (1965). Three parallel conducting systems in the stalk of a hydroid. *J. Exp. Biol. J. Exp. Biol.* **42**, 139–52.

LEGHISSA, S. (1950). L'evoluzione morfologica del tessuto nervoso nei Celenterati fissi. *Boll. Zool.* **17**, Suppl., 213–53.

LEGHISSA, S. (1951). Le reazioni nervose in *Tubularia. Mem. R. Acad. Bologna, Sci. fis.,* **8**, 29–46.

MACKIE, G. O. (1959). The evolution of the Chondrophora (Siphonophora-Disconanthae): new evidence from behavioural studies. *Trans. Roy. Soc. Can.* **53**, sect. 5, 7–20.

MACKIE, G. O. (1964). Analysis of locomotion in a siphonophore colony. *Proc. Roy. Soc.* B, **159**, 366–91.

MACKIE, G. O. (1965a). Conduction in nerve-free epithelia of siphonophores. *Amer. Zool.* (in the Press).

MACKIE, G. O. (1965b). Growth of the hydroid *Tubularia* in culture. *Symp. zool. Soc. Lond.* (in the Press).

MACKIE, G. O. & BOAG, D. A. (1963). Fishing, feeding and digestion in siphonophores. *Pubbl. Staz. zool. Napoli*, **33**, 178–96.

McCULLOUGH, C. B. (1962). Modification of *Hydra* pacemaker activity by light. *Amer. Zool.* **2**, 430.

McCULLOUGH, C. B. (1963). The contraction burst pacemaker system in hydras. *Proc. XVI Int. Cong. Zool.* vol. I, 25.

McCULLOUGH, C. B. (1965). Pacemaker hierarchies in *Hydra. Amer. Zool.* (in the Press).

NICOL, J. A. C. (1960). *The Biology of Marine Animals.* London: Pitman and Sons.

PANTIN, C. F. A. (1935). The nerve net of the Actinozoa. I. Facilitation. *J. Exp. Biol.* **12**, 119–38.

PANTIN, C. F. A. (1956). The origin of the nervous system. *Pubbl. Staz. zool. Napoli*, **28**, 171–81.

PARKER, G. H. (1919). *The Elementary Nervous System.* Philadelphia: J. B. Lippincott Co.

PASSANO, L. M. (1962). Neurophysiological study of the coordinating systems and pacemakers of hydras. *Amer. Zool.* **2**, 435–6.

PASSANO, L. M. & McCULLOUGH, C. B. (1962). The light response and the rhythmic potentials of hydra. *Proc. Nat. Acad. Sci., Wash.*, **48**, 1376–82.

PASSANO, L. M. & McCULLOUGH, C. B. (1963). Pacemaker hierarchies controlling the behaviour of hydras. *Nature, Lond.*, **199**, 1174–5.

PASSANO, L. M. & McCULLOUGH, C. B. (1964). Coordinating systems and behaviour in *Hydra.* I. Pacemaker system of the periodic contractions. *J. Exp. Biol.* **41**, 643–64.

PASSANO, L. M. & McCULLOUGH, C. B. (1965). Coordinating systems and behavior in *Hydra.* II. The rhythmic potential system. *J. Exp. Biol.* **42**, 205–31.

PEARSE, A. S. (1906). Reactions of *Tubularia crocea* (Ag.). *Amer. Nat.* **40**, 401–7.

PROSSER, C. L. (1962). Conduction in nonstriated muscles. *Physiol. Rev.* **42**, suppl. 5, 193–206.

REES, W. J. (1957). Evolutionary trends in the classification of capitate hydroids and medusae. *Bull. Brit. Mus. (nat. Hist.) Zool.* **4**, 455–534.

RUSHFORTH, N. B., KROHN, I. T. & BROWN, L. K. (1964). Behavior in *Hydra*: inhibition of the contraction responses of *Hydra pirardi. Science*, **145**, 602–4.

WOODBURY, J. W. & CRILL, W. E. (1961). On the problem of impulse conduction in the atrium. In *Nervous Inhibition*, ed. E. Florey, pp. 124–35. Oxford: Pergamon Press.