CONDUCTION AND CONTRACTION IN THE COLUMN OF HYDRA

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INTRODUCTION

Passano and McCullough, in a recent series of papers (1962, 1963, 1964, 1965; Passano, 1963), describe features of some spontaneously active co-ordinating systems in *Hydra*. One of these co-ordinating systems is remarkable in that it produces very large (up to 30–40 mV.) and long (200–500 msec.) electrical pulses as recorded with extracellular electrodes. This co-ordinating system was termed the contraction burst system because each of the electrical potentials is associated with shortening of the hydra's column and, in Passano and McCullough's experiments, the pulses usually occurred in bursts lasting 30–60 sec. and containing 5–12 pulses. Using an improved recording method in which the hydra is essentially unrestrained we have found that while in some animals the electrical pulses and column contractions occur principally in bursts in other animals they occur principally as single, widely spaced events (Rushforth and Josephson, unpublished). For this reason the electrical pulses will be here termed contraction pulses (CPs) rather than contraction burst pulses.

The column shortening associated with contraction pulses is due to contraction of longitudinal musculature in the column ectoderm. Since the nerve net of the column is also ectodermal (gastrodermal nerve cells are sparse and do not form a complete net; Semal-Van Gansen, 1952), Passano & McCullough (1965) suggested that the conducting system transmitting contraction pulses through the column might be the ectodermal nerve net. While the ectodermal nerve net might be responsible for the propagation of activity, the large size of the potentials indicates that their source must be something other than the small, scattered cells of the nerve net. Recently epithelial conduction has been clearly demonstrated in a siphonophore (Mackie, 1965) and there is suggestive evidence that it occurs in hydroids as well (Josephson, 1965). The demonstration of epithelial conduction in coelenterates raises the possibility that the CP system in hydra may be epithelial rather than nervous. The experiments described below were begun as part of an investigation on the properties of the CP system as a possible example of an electrically active epithelium. This paper considers the conduction velocity of the potentials through the column and the relation between the potentials and column contraction. A later paper (Josephson & Macklin, 1967) will present evidence that the CPs are indeed due to epithelial activity which can also be recorded as a potential change occurring across the body wall of the whole animal.

MATERIALS AND METHODS

The animals used were *Hydra littoralis*, grown under conditions similar to those described by Loomis & Lenhoff (1956). The hydras were fed daily on newly hatched *Artemia* and maintained in a culture solution containing $1 \cdot 5 \times 10^{-3}$ M-$CaCl_2$, $1 \cdot 2 \times 10^{-3}$ M-$NaHCO_3$, and $1 \cdot 2 \times 10^{-4}$ M-Na_4EDTA.

Electrical recordings were made with suction electrodes having bell-shaped openings, $0 \cdot 4$–$0 \cdot 6$ mm. in internal diameter at the open end and narrowing to $0 \cdot 15$–$0 \cdot 2$ mm. in internal diameter within $0 \cdot 5$–1 mm. of the open end. A syringe attached to the electrode by flexible tubing was used to create suction and hold the base or the hypostome of the hydra to the electrode. The distal portion of the electrode was filled with culture medium; electrical contact to the interior of the electrode being made with a chlorided silver wire inserted through the wall of the flexible tubing. A chlorided silver wire in the solution surrounding the animal served as an indifferent electrode. Generally the base of a hydra was held in one electrode and the hypostome in another. In some experiments column contraction was measured by attaching the basal electrode to the movable anode of an RCA 5734 mechanoelectric transducer (Josephson, 1966). The attachment of the electrode to the transducer was essentially non-compliant so contraction was measured isometrically. All experiments were done at 19–23° C.

The electrodes used are quite convenient for recording from hydra and their manufacture deserves description. Glass tubing of convenient diameter for available manipulators is heated and drawn until one end is about $0 \cdot 5$ mm. in internal diameter. The tubing is mounted vertically, drawn end down, and inserted through a heating coil consisting of a single loop of platinum wire. The lower end of the tubing should be about 1 cm below the coil. The tubing within the heating coil is observed with a microscope while current is passed through the coil. As the glass gets hot it flows together and the inner walls of the tubing bulge inward to form a constriction. The heating current is turned off when the internal diameter of the constriction is $0 \cdot 1$–$0 \cdot 2$ mm. The tubing is removed from the coil, scored with a diamond just distal to the constriction, and the distal end of the tubing is broken off at the score. The newly formed electrode tip is fire-polished in a small flame and the shank above the constriction is heated and bent to a convenient shape. *Hydra* culture solution has low conductivity and the electrical resistance of electrodes filled with culture solution is usually several million ohms. Threading a length of silver wire from the proximal end of the electrode to the constriction greatly reduces the electrical resistance, decreases electrode noise, and makes it possible to use amplifiers with relatively low input impedance.

Animals will feed and bud normally while being loosely held in a single, basal electrode and recordings can be made for apparently unlimited periods. Suspending an animal between two electrodes, however, does introduce difficulties. The presence of an electrode on the hypostome or the tension created when the animal contracts between two electrodes is apparently excitatory, for the frequency of column contractions is much greater with 2-electrode recordings than when the animal is held in a single basal electrode. The experiments with 2-site recording were generally terminated by the animal pulling free from one of the electrodes or tearing itself

into two pieces. Most preparations remained intact at least several minutes and the best preparations lasted for a few hours.

RESULTS

I. *Conduction in the column*

Initially the conduction velocity in the column was measured by recording from the base and the hypostome of a hydra, while it was stimulated with 1 msec. current pulses passed through the basal electrode. A bridge circuit similar to that illustrated by Frank & Becker (1964, p. 75) was used to stimulate and simultaneously record from the basal electrode. The two electrodes suspending the hydra were arranged so that the column became slightly slack when the animal relaxed completely.
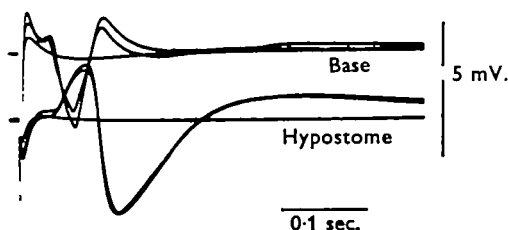

Fig. 1. Recording from the base and hypostome of a hydra while stimulating through the basal electrode. Inter-electrode distance = 3·3 mm. Positive is shown as down in this and following figures.

Two principal difficulties were encountered with this technique. First, the stimulus threshold is rather high, about 10^{-5}–10^{-6} A. with 1 msec. pulses, and bridge balance is difficult to achieve. Capacitative surges of current during the current pulse tend to obscure early portions of the potential from the base. Secondly, the shapes of the recorded pulses are different at the two sites. This makes determining the latency between equivalent events at the base and hypostome difficult, for it is not obvious which parts of the pulses recorded at the two sites are equivalent. At the stimulated base the potentials are typically triphasic, with a small negative deflexion followed by a large positive peak and in turn followed by slow, negative component. At the hypostome the response is also typically triphasic, but the initial negative deflexion is much larger and longer than at the base (Fig. 1). Deviation from these patterns is often seen. Sometimes the initial negativity at the hypostome is reduced or absent, and frequently pulses contain several peaks and notches, suggesting a compound origin. Differences in pulse shapes at the base and hypostome result from different latencies at the two sites and from the fact that activity leaves the basal electrode while it travels into the hypostomal electrode. Evidence presented below indicates that the positive peak at each site is the most appropriate portion of the response for conduction velocity measurements. Conduction velocities for several animals, computed from time differences between positive peaks at base and hypostome, are shown in Table 1. The distance between recording electrodes was measured to the electrode constrictions.

Outward current from the holding electrode is slightly more effective in activating the CP system than is inward current, the threshold for outward current being lower

by 10–25 %. Like some other coelenterate conducting systems (Josephson, 1961, 1965) the CP system in hydra frequently fires repetitively following single stimuli, especially if the stimulus intensity is much above threshold.

There is no doubt that the large potentials evoked by stimuli are the same as the CPs which occur spontaneously. Column shortening is correlated in a 1:1 fashion with both spontaneous and evoked potentials. Spontaneous and evoked potentials are similar in size (a few to several tens of mV. in different preparations) and often in shape. An evoked potential and a number of apparently spontaneous pulses are

Table 1. *CP conduction velocity–column stimulated through basal holding electrode*

Animal no.	Inter-electrode distance (mm)	Conduction velocity ± S.E. (cm./sec.)	n
1	2·5	3·9 ± 0·3	4
2	3·1	5·7 ± 0·2	36
3	3·9	5·1 ± 0 2	6
4	4·3	4·2 ± 0·1	15
5	6·5	5·2 ± 0·1	16

Conduction velocities were computed from the time interval between positive peaks at the basal and hypostomal electrodes. n = number of determinations with each animal.
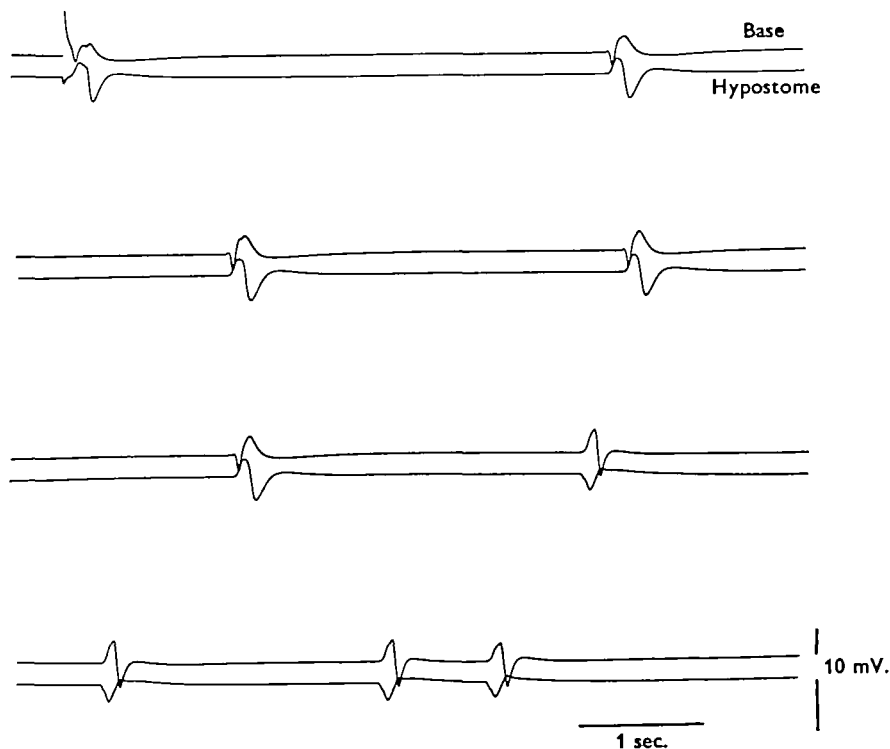

Fig. 2. Repetitive firing following stimulation through a basal electrode. Inter-electrode distance = 6·5 mm. Note the apparent shift in originating locus following the fifth pulse, marked by a change in leadership to the hypostomal electrode and a change in pulse shape at both electrodes.

compared in Fig. 2. The first four of the spontaneous pulses apparently originated near the base, for they appear first at the basal electrode and their shape is strikingly similar to the pulse initiated at the base by the stimulus. In the middle of the train the initiating locus shifted distally and the pulses began appearing first at the hypostomal electrode. This change in origin is also reflected in changes in the pulse shapes at the two electrodes. This record indicates that pulses can be spontaneously initiated at the base of the hydra, but it does not preclude the possibility that in unrestrained and unstimulated animals the initiating locus is in the hypostomal region as was suggested by Passano & McCullough (1964).
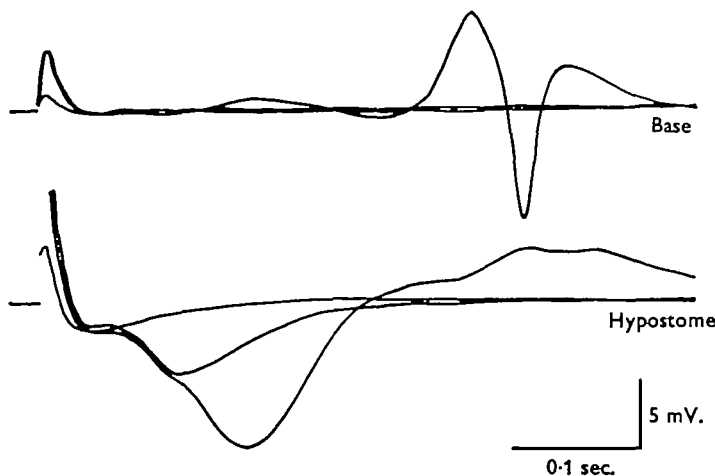

Fig. 3. Activity recorded from the base and hypostome of a hydra during column stimulation close to the hypostomal electrode. The records were photographically superimposed and traced. Distance between recording electrodes = 7·9 mm.; stimulating electrode 1·7 mm. from hypostomal electrode. Note that one stimulus evoked a response at the hypostome which was not conducted to the basal electrode but a later stimulus produced a larger response at the hypostome which was conducted to the base.

A different technique was used in later velocity measurements to minimize the difference in pulse shapes at the two recording electrodes. The animal was suspended between two electrodes as before but it was stimulated at different points on the column with a third electrode. To stimulate the animal the tip of a 0·1 mm. diameter silver wire, insulated to the tip, was placed against the desired point on the column and 1 msec. current pulses were passed between this electrode and a similar one a few millimetres away. Since activity is initiated in the central region of the column it travels toward both recording electrodes rather than toward one and away from the other as in the bridge stimulation experiments.

Non-propagated electrical responses were occasionally seen during these experiments. An example of a local response is shown in Fig. 3. The stimuli were delivered to the column near the hypostomal electrode. The first stimulus was ineffective; the second initiated an electrical response seen at the hypostomal electrode but not at the basal electrode; and the third stimulus evoked a larger response at the hypostome which was propagated to the base. The stimulus intensity and position were the same for each trial; the changing stimulus effectiveness was probably due to small movements of the hydra with respect to the stimulating electrode.

In most trials the recorded electrical response at the base and hypostome consists of an initial negative-going potential which is interrupted after some time by a positive-going response culminating in a positive peak. The initial negativity often begins almost immediately with the stimulus at both recording electrodes no matter where the column is stimulated. The delays to the onset of the positive slope and to the positive peak, on the other hand, vary systematically with the position of the stimulating electrode, becoming longer as the stimulating electrode is moved away
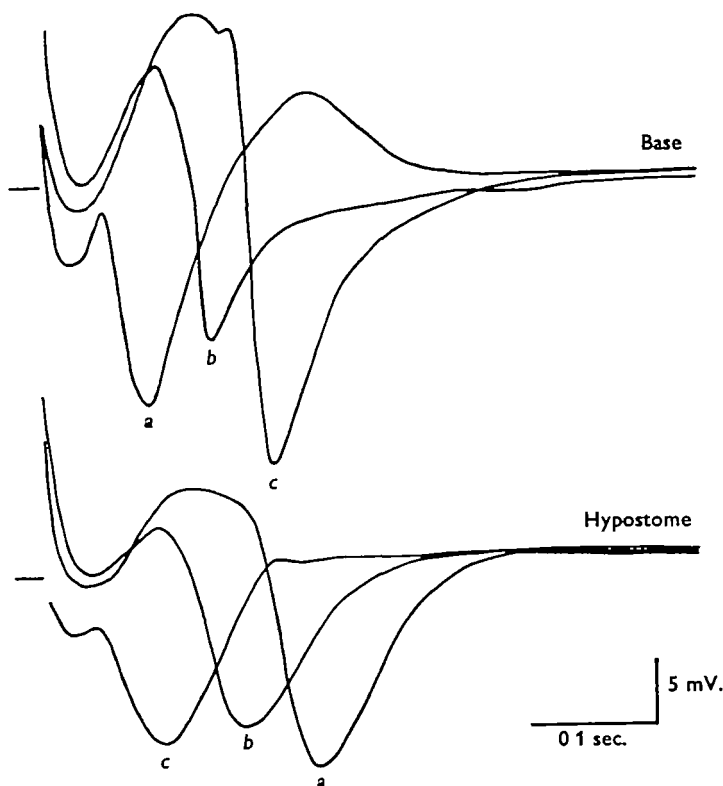

Fig. 4. Pulses recorded from base and hypostome during column stimulation at different levels. The traces were photographically superimposed and traced. Distance between recording electrodes = 7·8 mm. Stimulating electrode 1·25 mm. (a), 3·75 mm (b), and 6·25 mm (c) from the basal recording electrode.

from the recording site (Fig. 4). This behaviour indicates that activity in the column distant from a recording site is seen as a negative potential, while the arrival of activity at the recording electrode is signalled by a positive deflexion. For this reason the latency between the stimulus and the positive potential peak was used for conduction-velocity measurements in this series and in the bridge-stimulation series earlier described.

The method for determining conduction velocities with a moving stimulating electrode is illustrated in Fig. 5. Table 2 gives the conduction velocities determined for the six best preparations, selected on the basis of the number of determinations made before the animal pulled apart and the repeatability of latency determinations

at the same site. The values obtained, 3–8 cm./sec., agreed well with those from the bridge-stimulation experiments. One very interesting feature which emerges is that the basal and hypostomal conduction velocities are generally not equal, and the faster direction varies from animal to animal. The unequal conduction velocities may reflect asymmetries in the shapes of conducting cells or in their spatial organization.
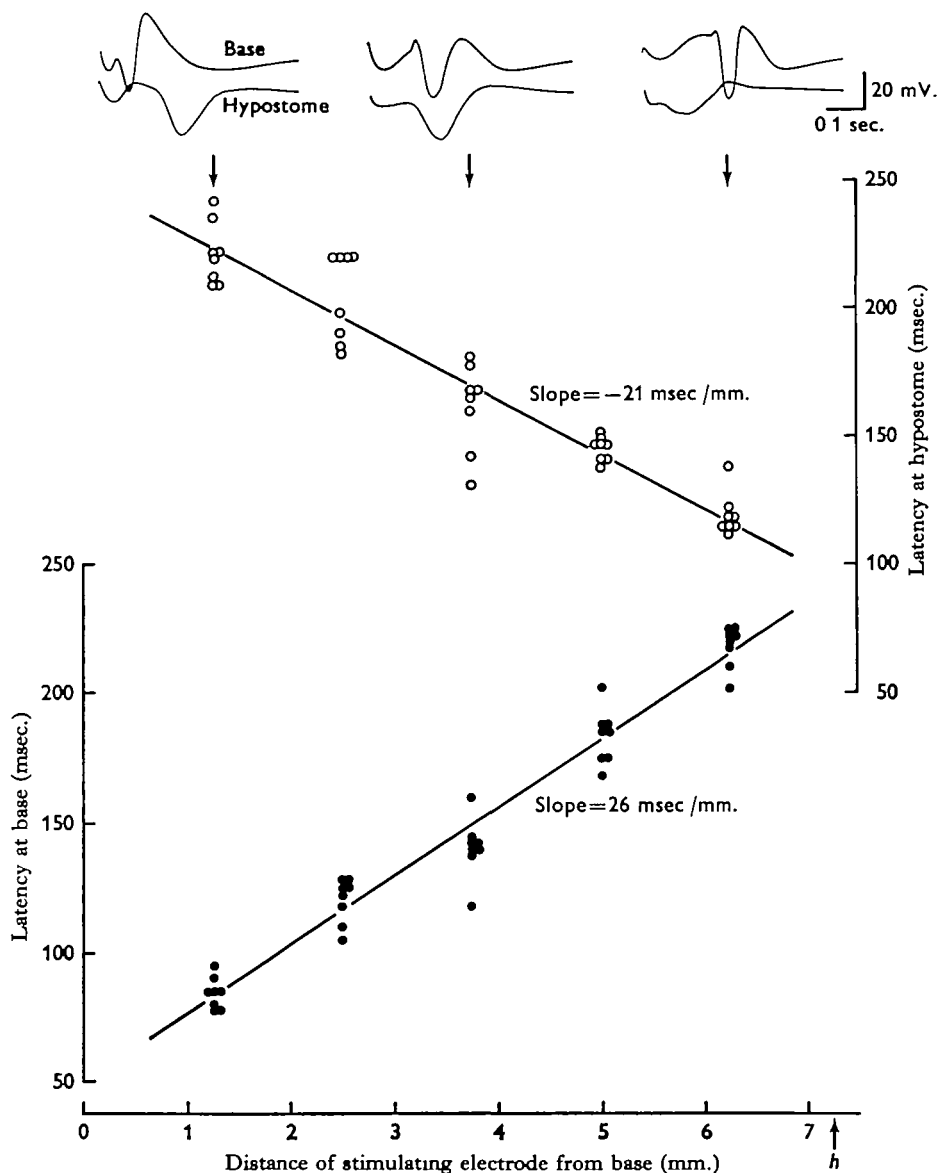

Fig. 5. The latency between a stimulus to the column and the appearance of the positive peak of the evoked potential at the basal and hypostomal electrodes. *h* indicates the position of the hypostomal electrode.

Table 2. *CP conduction velocity—column stimulated at several points
along its length*

Animal no.	Inter-electrode distance (mm.)	Basal conduction velocity (cm./sec.)	Hypostomal conduction velocity (cm./sec.)
1	7·75	5·2 (4·7–5·8, 35)	4 0 (3·6–4·4, 35)
2	8·30	5·2 (4·8–5·7, 38)	6·5 (5·3–8·3, 36)
3	7·25	4 7 (4·3–5·2, 14)	8·1 (6·4–11·1, 14)
4	5·33	5·8 (4·6–7·7, 18)	3·4 (2·8–4·5, 18)
5	6·25	3·8 (3·3–4·5, 18)	6·8 (5·1–10·0, 18)
6	7·25	3·8 (3·6–4·1, 40)	4·8 (4·3–5·4, 40)

Conduction velocities were computed from the slopes of lines like those of Fig. 5. The bracketed figures following each velocity are the 95 % confidence interval for the velocity and the number of points from which the slope was determined. In one case the number of points is different for basal and hypostomal velocities because at some stimulating positions near the hypostome the shock artifact obscured the response from the hypostomal electrode. The difference between basal and hypostomal velocities is statistically significant in each case ($P < 0.05$).

II. *Contraction of the column*

The column of a hydra suspended between two electrodes behaves as a viscous element. If the animal is stretched until it is just taut the column lengthens with time until after some minutes it hangs somewhat loosely between the electrodes. The slow lengthening is not uniform and is interrupted at intervals by spontaneous column contractions. The slow lengthening introduces no major difficulties in conduction-velocity measurements for the column reaches a more-or-less stable configuration when it is slightly slack. The lengthening under tension, however, does make it difficult to measure the tension produced by column contraction. With the recording method used single column contractions or the first few contractions of a burst produced no measurable tension in a slack animal but merely took up the slack. To avoid this the inter-electrode distance was periodically increased during contraction measurements so that the column was always under slight tension. As the length of the column was progressively increased the column became thinner, and the contractions grew weaker. All these experiments were eventually terminated by the column pulling into two pieces.

The column contractions associated with CPs frequently occur with two distinct phases (Fig. 6). There is an initial increase in tension which reaches a peak about 1 sec. after the onset of contraction. This initial tension rise is typically followed by a partial relaxation and then a second, rather slow tension increase which reaches a broad maximum 5–10 sec. after the initiation of contraction. Closely spaced contractions sum. When the electrical potentials are separated by less than about 0·5 sec. the contractions smoothly fuse, at longer intervals the individual contractions appear as distinct steps of tension increase. The slow tension component is most pronounced following single, widely spaced CPs. Relaxation from the higher tension levels reached during summed contractions is more rapid than that following single potentials and the relaxation often shows little or no indication of a slow contraction phase (Fig. 6B). Column contraction was first detected 0·1–0·15 sec. after the onset of the electrical potential which usually appeared first from the hypostomal electrode in these experiments.
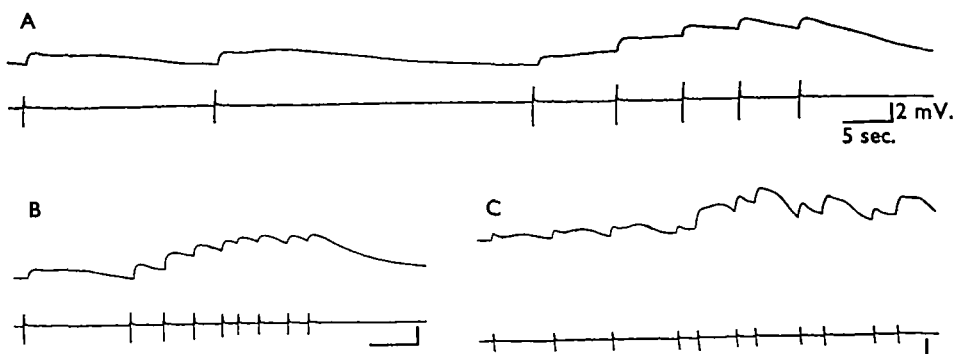
Fig. 6. Spontaneous potentials (lower traces) and contractions (upper traces) in the column of hydra. Note that C is on a slower time base than A and B.

DISCUSSION

Passano & McCullough (1964) determined the conduction velocity of the contraction pulse system in *Hydra pirardi* by recording at two sites on the column during spontaneous CP firing in whole animals and in one animal which was longitudinally split at the distal end to create a longer conduction pathway. They reported the conduction velocity to be 15 cm./sec. The difference between this value and that found in the present study with *H. littoralis* (about 5 cm./sec.) seems rather large to be ascribed to a species difference or to a difference in the way the animals were handled. It is possible that the different values for conduction velocity reflect different methods used to measure the pulse transit time between two recording electrodes. Passano & McCullough do not indicate how they measured the time interval between events at the two recording sites. If their measurements were made to the onset of activity in each channel, the apparent transit time between electrodes would be shorter and the computed conduction velocity greater than if they had measured to the potential maximum as was done here. The CPs are quite large in amplitude, and current flow about an active area can produce significant potential changes in adjacent inactive regions. In *H. littoralis*, which is smaller than *H. pirardi* and in which the interelectrode distance is necessarily quite small, potential changes are often recorded with a basal or hypostomal electrode almost immediately after stimulation even when the activity is initiated at the opposite end of the animal. The arrival of the activity at an electrode, as judged by the change from a negative to a positive potential, can follow the onset of a potential change at that electrode by 100 or more milliseconds. Thus the onset of a potential change occurs considerably sooner than the arrival of the active area at the recording electrode, and using the onset underestimates the pulse conduction time.

The CP system of hydra is in several ways similar to the conducting system found in the stalk of *Cordylophora* (Josephson, 1961) and to the slowest conducting system in the stalk of the hydroid *Tubularia* (Josephson, 1965). All three produce large electrical potentials, conduct slowly (3–5 cm./sec.) and tend to fire repetitively following brief electric shocks. In hydra it is clear that the potentials are of epithelial origin (Josephson & Macklin, 1967). The large size of the potentials from the

similar conducting systems in *Cordylophora* and *Tubularia* suggest that these two originate in epithelial activity rather than from fine, dispersed nerve fibres, the organization of neurones generally found in coelenterates. But identifying the source of the potentials as epithelial does not necessarily mean the propagation of activity is also a property of an epithelium. It is possible that the epithelium does not itself conduct but rather it is activated by a wave of excitation conducted in adjacent nerve elements. Conclusively identifying the cellular elements conducting activity in tissues containing both nervous and non-nervous cells is likely to be a difficult task.

I can think of three explanations which might be offered to account for the presence of both a rapid and a slow component in the contraction of a hydra's column:

(1) The biphasic contraction might be an inherent property of the longitudinal musculature itself, due to either contraction of two populations of longitudinal muscle fibres with different temporal characteristics or to biphasic contraction of individual fibres.

(2) The dip in the tension curve separating the peaks of the two apparent components might be due to a relatively brief and slightly delayed contraction of circular muscle elements transiently antagonizing the effects of longitudinal muscle contraction.

(3) If the longitudinal musculature were excitable by tension increase the slow component might be explained as being a contraction initiated by the tension produced during the initial rapid contraction.

Of these three possibilities only the last can be directly eliminated. During these experiments, the distance between the holding electrodes was periodically increased to compensate for slow lengthening of the column and to hold the animal under slight tension. Occasionally stretching the column initiated a CP and column contraction, but when CPs were not initiated no active column contraction was seen following stretch, even when the tension increase in the column was greater than that produced by normal single contractions. This was true if the column was stretched during quiescent periods, while it was contracting, or while it was relaxing following contraction. Apparently, longitudinal muscle contraction is not initiated by sudden increase of the tension on the muscle, at least not in the tension range produced by its own activity during single contractions. The second possibility, that of a tension record complicated by simultaneous activity of antagonistic muscles, cannot be directly eliminated but seems unlikely. The contractions recorded were spontaneous, initiated by normal processes in the animals. It is improbable that the longitudinal and circular muscles are programmed to contract simultaneously, for simultaneous contraction would reduce the effectiveness of either muscle set in changing the shape of the animal and would add only an increase in the internal hydrostatic pressure. Thus the biphasic contraction is probably a property inherent to the longitudinal musculature itself.

Muscles which show both fast and slow contractions are fairly common in invertebrates and have been described in coelenterates, annelids, arthropods and molluscs (Hoyle, 1962). In most cases the fast and slow contractions are activated by different pathways and it is generally possible to excite either alone by appropriate adjustment of the strength, duration, or location of activating electrical stimuli. For example, preparations from the gut of the clam *Spisula* can give contractions closely resembling

those of hydra column with an initial phasic component and a more slowly rising tonic component (Prosser, Nystrom & Nagai, 1965). The two components of *Spisula* gut contraction have intersecting strength-duration curves and can be excited independently; the phasic component has the lower threshold with short stimuli and the tonic component has the lower threshold with long stimuli. In coelenterates fast and slow contractions of the same muscle have been best studied in sea anemones. The available data about pathways initiating the two kinds of response are somewhat contradictory. It is reported that the thresholds for fast and slow contraction are the same in whole animals and in isolated muscle preparations (Batham & Pantin, 1954; Pantin, 1965), suggesting that the two responses are activated by a common mechanism. On the other hand, slow contractions propagate much more slowly than do fast responses (Pantin, 1965), suggesting two activating pathways. The longitudinal musculature in the column of *Cerianthus* gives fast and slow contractions resembling those of sea-anemone muscles, and here there is rather clear evidence for separate activating pathways. Excitation initiating quick responses will spread through a narrow bridge of tissue joining two muscle strips while the slow contractions are restricted to the stimulated strip (Horridge, 1958). The fact that the fast and slow contraction components in hydra appear during single spontaneous contractions suggests a common activating pathway, for it seems unlikely that spontaneous activity would initiate simultaneous firing in two independent pathways.

It might be pointed out that the slow contraction component in hydra is unlike slow contraction of anemone muscles in that it is most conspicuous following single firings in the conducting system and declines during repetitive firing while the slow contractions of anemone muscles require repetitive stimuli for their initiation.

SUMMARY

1. A suction electrode is described which allows long-term recording of spontaneous activity from essentially unrestrained *Hydra*. These electrodes were used to hold the animal while measuring column contractions and to measure the conduction velocity of the electrical potentials (contraction pulses = CPs) associated with contraction.

2. CPs are conducted in the column at 3·4–8·1 cm./sec. The conduction velocity generally differs in the basal and hypostomal directions but the direction of faster conduction varies from animal to animal.

3. The latency between CPs and measurable column contraction is 0·1–0·15 sec. During isometric contractions following single CPs there is a rapid tension rise reaching a peak in about 1 sec. which is usually followed by partial relaxation and then a second, slower tension rise. Available evidence indicates that both tension components are due to contraction of longitudinal musculature and both are activated by the same conducting system.

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