NEUROMUSCULAR MECHANISMS
OF BURROW IRRIGATION IN THE ECHIUROID WORM
URECHIS CAUPO FISHER & MACGINITIE

II. NEUROMUSCULAR ACTIVITY OF DISSECTED PREPARATIONS

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INTRODUCTION

In an earlier paper anatomical description of the neuromuscular system provided a basis for behavioural and physiological investigations of intact animals (Lawry, 1966). This report presents results from a study of dissected preparations in which pacemakers were localized, perfused with drugs and stimulated electrically in order to investigate the interaction of the musculature and nervous system during the production and propagation of the peristaltic waves employed in burrow irrigation.

METHODS

Unanaesthetized *Urechis* were incised mediodorsally or bisected transversely and pinned to paraffin-lined dissecting dishes (Figs. 1, 2). Contractions of the body-wall were recorded kymographically by means of light isotonic levers attached by hairs to small hooks in the muscle layers. Electrical or tactile stimulation was applied to the preparations. The behaviour of specimens perfused with body fluid was similar to that of preparations in sea water, so sea water was used to make up drug solutions and to bathe experimental animals; these manifested normal peristaltic movements for several hours if kept at 13° C. A 0.18×10^{-3}M solution of acetylcholine (ACh) in sodium dibasic phosphate citric acid buffer, a 0.21×10^{-4}M solution of epinephine, a 0.64×10^{-3}M buffered solution of eserine, and a 0.40×10^{-3}M solution of 5-hydroxytryptamine (5-HT) were used to make 10^{-4}, 10^{-5} and 10^{-6}M solutions in sea water. Sea water was saturated with oxygen and nitrogen, and these solutions were kept on crushed ice and warmed to 13° C. before being applied to the preparations. Peripheral nerves were isolated for extracellular recording by detaching them either proximally or distally and drawing them into small pools of oil on silver hooks. Electrical activity was displayed and recorded by conventional means, and an RCA 5734 mechanical transducer was used to record muscle tension.

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RESULTS

(1) *Localization of the anterior pacemaker*

Observations of intact worms (Lawry, 1966) indicated that waves of peristalsis which move water through the burrow first become visible 5 mm. behind the point at which the proboscis joins the trunk (Fig. 3). Presumably the anterior pacemaker
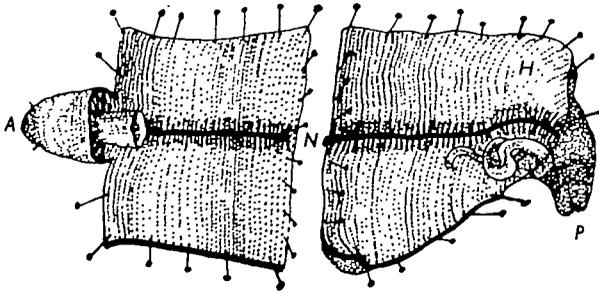

Fig. 1. Dissected *Urechis caupo* pinned ventral side down in a dissecting dish. *P*, Proboscis; *N*, nerve cord; *A*, anus; *H*, position for hook for kymograph records.
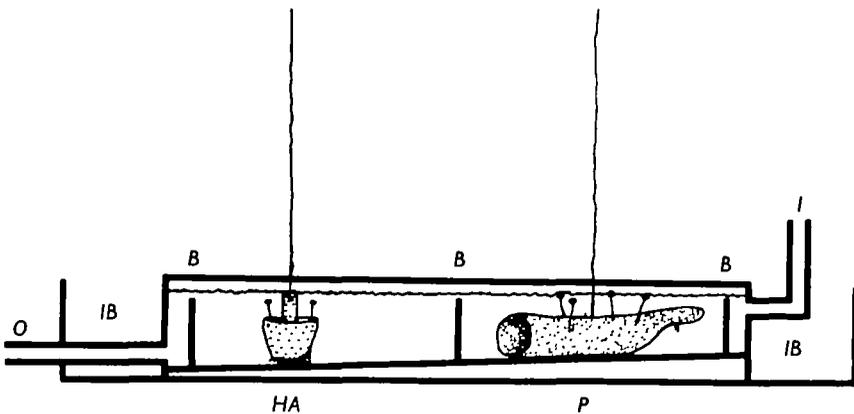

Fig. 2. Recording apparatus for pharmacological studies. *O*, Outlet; *IB*, ice bath; *B*, baffle; *HA*, hind-gut–anus preparation; *P*, proboscis preparation; *I*, inlet.
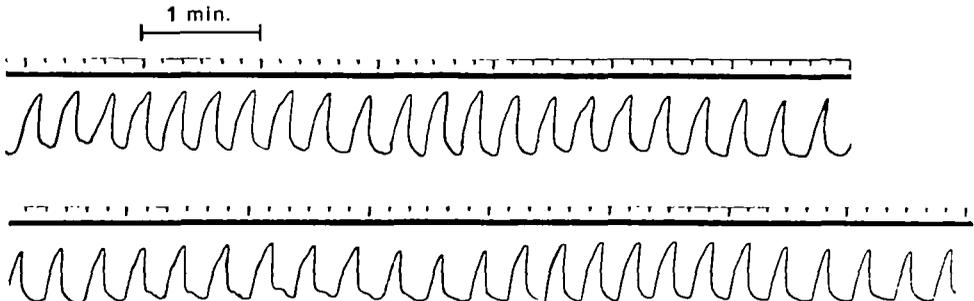

Fig. 3. Kymograph record of peristaltic waves produced by anterior pacemaker of *Urechis*. The smaller divisions of the timer trace indicate intervals of ten seconds. There is no time lapse between the two records.

initiating these waves is localized in the region of the circumpharyngeal connectives, because the proboscis of a worm from which the circumpharyngeal connectives have been dissected displays no spontaneous activity and does not generate waves of peristalsis. Worms from which the circumpharyngeal connectives and proboscis have been removed by a transverse cut show after several minutes regularly propagating waves of peristalsis which appear to originate in the segment innervated by the cord immediately posterior to the cut. When the nerve cord is removed from the preparation described above after severing the peripheral nerves, no waves of peristalsis appear,
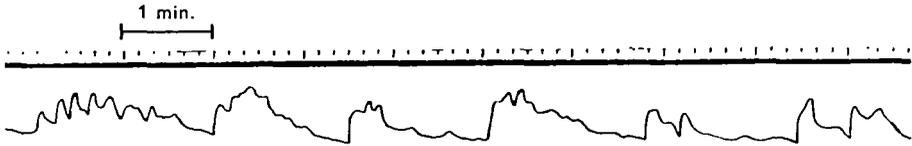

Fig. 4. Kymograph record of peristaltic waves propagated into proboscis region from posterior pacemaker. The anterior pacemaker was inactive, and the timer trace and recording system are the same as in Fig. 3.
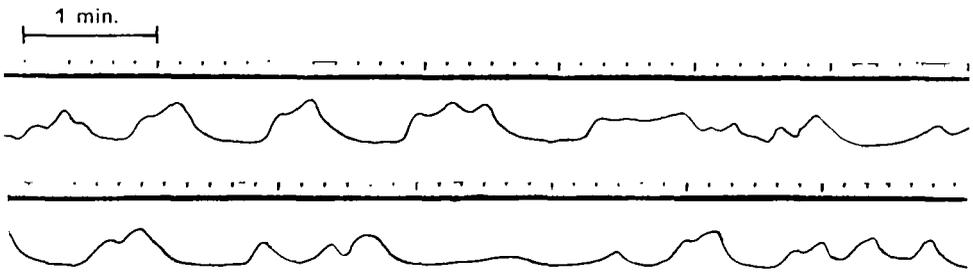

Fig. 5. Kymograph record of anal breathing movements recorded from the hind gut. The lever system is described in the text. There is no time lapse between the two records.

although the body-wall can still respond to tactile stimuli with a local, non-propagated contraction. The anterior pacemaker is sensitive to acetylcholine in concentrations of 10^{-6} M. This response is potentiated by eserine and consists of an increase in the frequency and amplitude of the peristaltic waves as recorded kymographically (Fig. 9). The proboscis region can be invaded by conducted activity produced by electrical stimulation of the nerve cord. Such invasion can reset the pacemaker rhythm (Fig. 14).

(2) *Localization of the posterior pacemaker*

The posterior pacemaker producing the anal pumping movements of *Urechis* appears to be located in the hind gut. Under normal circumstances an anal pulse travels several centimetres anteriorly, where it collides with and is obliterated by a descending pulse from the proboscis. Some anal pulses may progress to the anterior end of the worm if they are not met by a descending pulse (Fig. 3). Records of anal pulses made from the hind gut (Figs. 5, 8) indicate that several small inhalations occur in a minute, and exhalant movements appear as slightly larger peaks in the record every 2 or 3 min. The most posterior portion of an *Urechis* will exhibit regular peristaltic pumping movements for several hours. If, however, the nerve cord is dissected

out of this preparation, leaving the hind gut attached to the body-wall by its anal margin, the hind gut continues to move normally but the body-wall does not pulsate. If the hind gut is then removed leaving the nerve cord and body-wall intact, the hind gut continues to contract rhythmically, but the body-wall does not exhibit regular movements. Some irregular movements occur in preparations in which small pieces of hind gut remain attached to the body-wall.
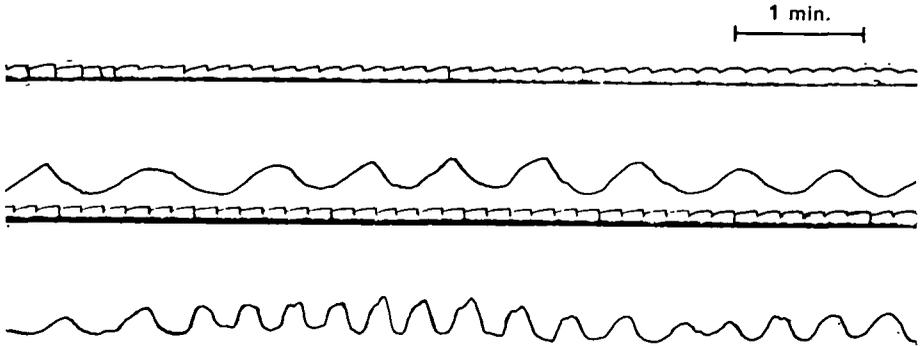
Fig. 6. The response of the proboscis in 10^{-6}M ACh. Twelve minutes elapsed between upper and lower records. 10^{-6}M ACh added at vertical line in lower record. Temperature was 13° C.
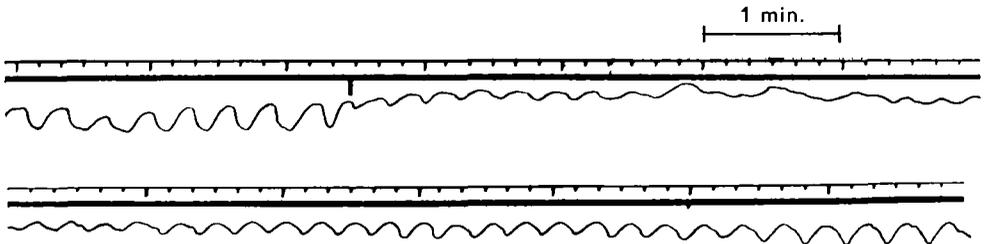
Fig. 7. The response of the proboscis in 10^{-5}M ACh. No time elapsed between the two records. 10^{-5}M ACh added at vertical bar in first record. Temperature was 11° C.
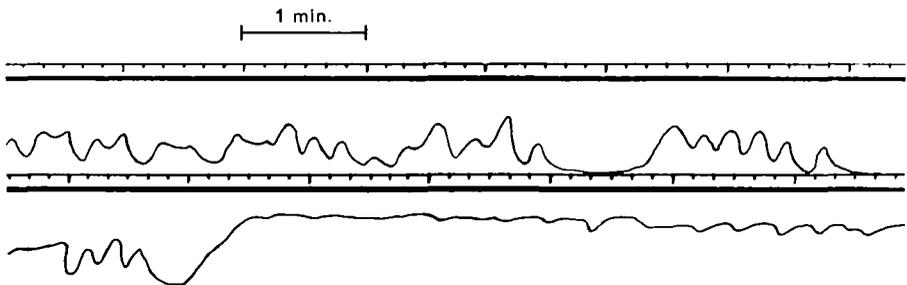
Fig. 8. The response of the hind gut–anus preparation in 10^{-5}M ACh. Seven minutes elapsed between upper and lower records. 10^{-5}M ACh added at vertical mark in lower record. Temperature was 11° C.

(3) Interactions between body-wall and nerve cord

Posteriorly directed waves of contraction in the layers of circular muscle were initiated in the first few millimetres posterior to cuts which isolated the proboscis from the trunk of dissected preparations. Pieces of nerve cord and body-wall showed

posteriorly propagating waves of peristalsis, commencing at their anterior ends, if the pieces were longer than about 30 mm. Pieces shorter than 25 mm. rarely showed spontaneous waves, although they could at times be made to contract by tactile stimulation of the body-wall. Preparations in which the body-wall was transected and pinned carefully, leaving the cord connecting the two pieces unstretched and intact, showed no decrement in the velocity of posterior conduction of a wave of peristalsis originating from the anterior end.

Preparations in which the cord was transected leaving the body-wall intact gave ambiguous results. Sometimes it was obvious that a second pulse was generated in the posterior segment of body-wall at the same time as a descending pulse arrived at the cut; on other occasions, the phasing between the arrival of a descending pulse at the cut and the initiation of a pulse posterior to it was so irregular that the latter appeared to be an independent event.

Preparations were made in which the nerve cord in the middle region of the trunk was detached for a few millimetres from the body-wall by cutting the peripheral nerves; the isolated segments of cord thus produced were suspended on pins above the body-wall. In these animals waves of contraction did not pass the raised sections of cord, but pulses which propagated posteriorly were generated on the posterior side of the raised segment. At first these pulses appeared synchronized with the pulses arriving at the raised segment from the proboscis, but after a few minutes the pulses generated posteriorly lagged behind those from the proboscis and subsequently showed independent phase relations.

Parasagittal cuts through the body-wall musculature about 15 mm. lateral and parallel to the nerve cord confined the propatating ring of contraction to the musculature medial to the cut. The musculature lateral to the cut remained uncontracted and showed no signs of spontaneous activity.

In preparations from which two simultaneous kymograph tracings were recorded from anterior and posterior ends of the body-wall, cuts in the body-wall or nerve cord between the hooks served to desynchronize the two traces. Posterior segments of nerve cord and body-wall usually pulsated more slowly when isolated than anterior segments. Cuts made in the body-wall or nerve cord in the posterior regions of dissected preparations had little effect on the rate of the anterior pacemaker until they were made within about 50 mm. of the proboscis; at this distance cuts were followed by acceleration in the rate of pulse initiation from the anterior pacemaker. Table 1 gives the results of two typical experiments.

The effects of drugs and dissolved gases

Preparations were perfused with suspected neuro-transmitters or substances known to modify neuromuscular activity of other invertebrates. In the presence of 10^{-6}M ACh the frequency of peristaltic waves initiated behind the proboscis almost doubled (Fig. 6). When the concentration of drug was increased to 10^{-5}M, the glands at the base of the proboscis exuded large quantities of mucus; the higher frequency was maintained, superimposed upon a slight tonic contracture of the musculature of the body wall (Fig. 7). The rather irregular contractions of a hind gut–anus preparation became more regular when the preparation was exposed to 10^{-5}M ACh, and a tonic contracture of the hind gut musculature occurred (Fig. 8). 10^{-5}M Eserine also slightly

Table 1. *Results of two typical experiments showing that cuts made in the body-wall and nerve cord of a dissected preparation within 50 mm. of the proboscis are accompanied by an accelerated rate of pulse initiation from the anterior pacemaker*

10 min. interval	No. of peristaltic waves*	10 min. interval	No. of peristaltic waves*
1	25	1	19
2	19	1	24
3	20	3	24
4	20	4	21
5	22	5	18
6	21	6 C	19
7	24	7	40
8	25	8	38
9 A	32	9	39
10	43	10	30
11	33	11	39
12	35	12	32
13	34		
14	32		
15	33		
16 B	34		
17	44		
18	61		
19	50		
20	49		
21	41		
22	60		
23	55		
24	54		
25	62		

A. Anterior 60 mm. of worm severed by transverse cut from the rest of trunk.
B. Anterior 50 mm. of worm severed by transverse cut from rest of trunk.
C. Anterior 50 mm. of worm severed by transverse cut from rest of trunk.
* Recorded in interval from anterior end of preparation by means of a kymograph.
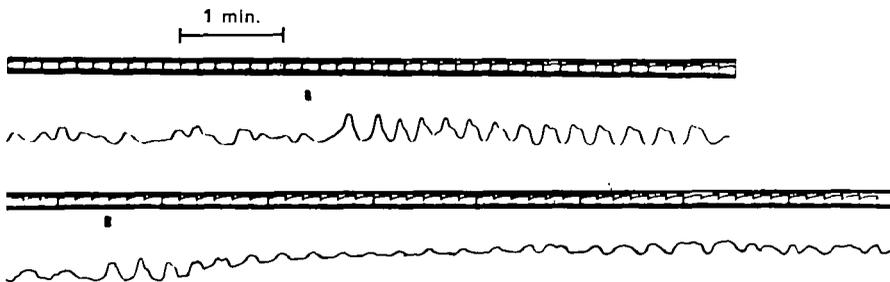
1 min.

Fig. 9. The response of the proboscis in eserine and ACh. Twenty-eight minutes elapsed between first and second records. 10^{-5}M eserine added at vertical mark in first record. 10^{-4} ACh added at vertical mark in second record. Temperature was $13°$ C.

accentuated the activity of proboscis and hind gut–anus preparations (Figs. 9, 10), and when combined with 10^{-5}M ACh appeared to potentiate its effect on both preparations. When 10^{-6}M ACh was added to an eserinized hind gut–anus preparation, a contraction of the hind gut musculature was followed after 20 min. by a cessation of the exhalant contractions (Fig. 10). The addition of 10^{-5}M ACh was accompanied by a further contracture of the hind gut musculature, and the amplitude of the anal

movements superimposed upon this contracture was depressed. The frequency of propagated peristaltic contractions initiated behind the proboscis in a preparation perfused with 10^{-5}M epinephine was approximately doubled (Fig. 11), whereas a
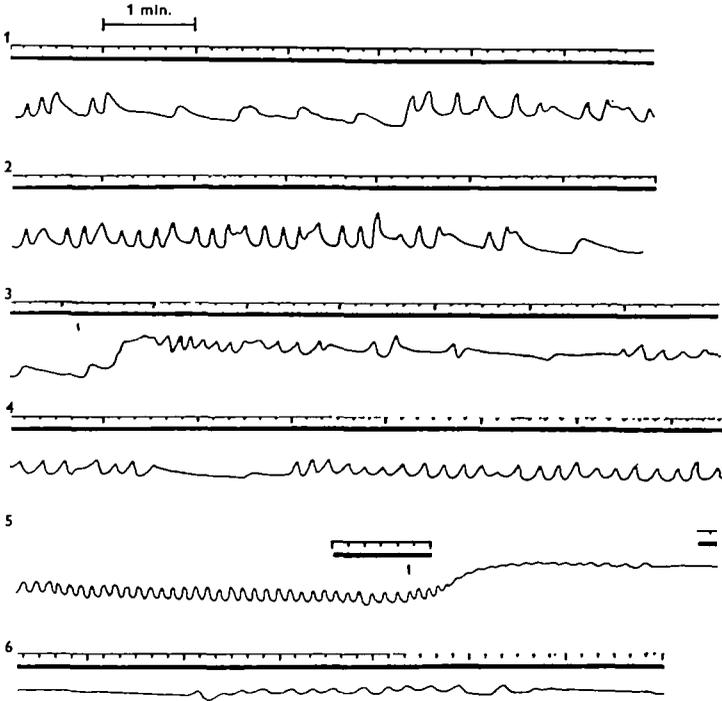
Fig. 10. The response of the hind gut–anus preparation in eserine and ACh. Thirty-eight minutes elapsed between the fourth and fifth record; all others are sequential. 10^{-5}M eserine added before commencement of first record; 10^{-6}M ACh added at vertical mark in the third record. 10^{-5}M ACh added at vertical mark in the fifth record.
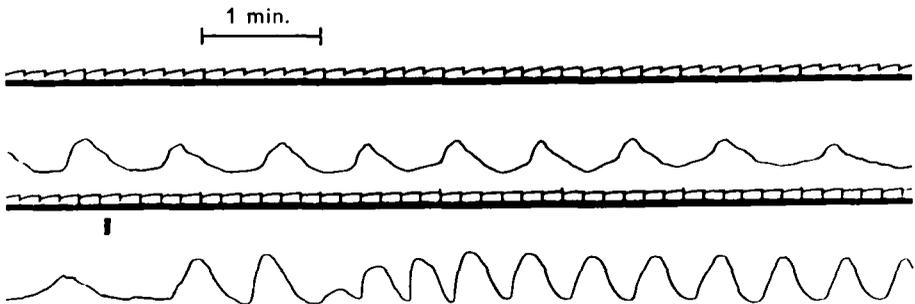
Fig. 11. The response of the proboscis pacemaker to 10^{-5}M adrenaline. Ten minutes elapsed between upper and lower records. 10^{-6}M adrenaline added at vertical mark in the lower record. Temperature was 13° C.

hind gut–anus preparation exhibited little change in activity. 5-HT had no observable effect upon these preparations. Nitrogenated sea water had little effect, but in oxygenated preparations the amplitude and frequency of pulses initiated at the proboscis were increased (Fig. 12), and the anal pulsations became more rapid (Fig. 13).

(5) Neurophysiological investigations

Resetting of the anterior pacemaker was effected by single electrical stimuli applied through electrodes implanted in the proboscis (Fig. 14), postponing the next spontaneous contraction by an interval equal to the length of the normal cycle. Such stimuli
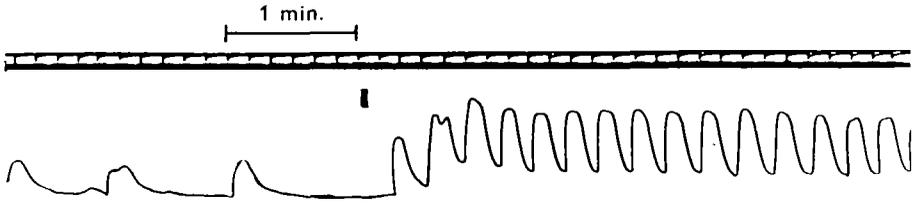
1 min.

Fig. 12. The effects of oxygen on the proboscis. Oxygenated sea water added at vertical mark in record; prior to this time preparation had been perfused with nitrogenated sea water. Temperature 11° C.
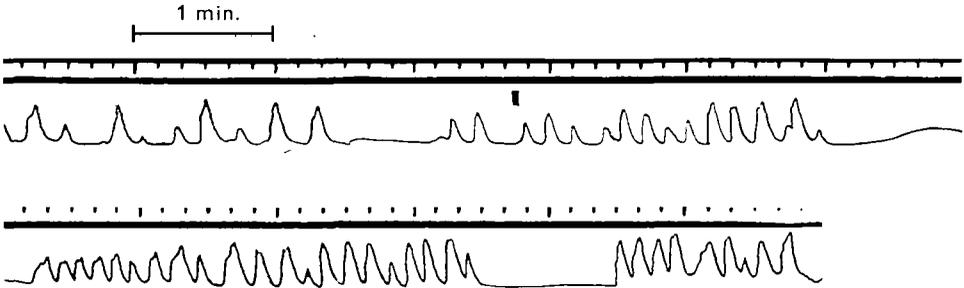
1 min.

Fig. 13. The effects of oxygen on the hind gut–anus preparation. Oxygenated sea water added at vertical mark in upper record; prior to this time preparation had been perfused with nitrogenated sea water. Temperature was 11° C.
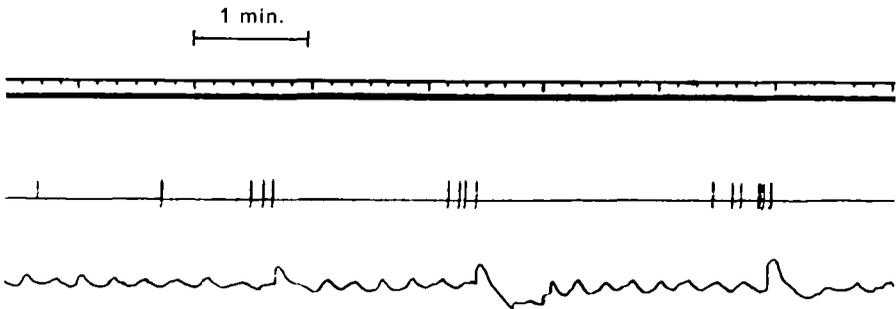
1 min.

Fig. 14. Reset of proboscis pacemaker during electrical stimulation of the proboscis. Stimuli of 3 V. and 5 msec. duration were delivered at various times during the pacemaker cycle. The second trace shows when shocks were applied; the third is a kymograph record of the body wall contractions.

sometimes produced momentary contraction of the body-wall. Repetitive stimulation of the nerve cord in the region of mid trunk was accompanied by tetanic contraction of the musculature of the body-wall, a marked shortening of the worm, and suppression of peristalsis. Rhythmic contractions reappeared after several minutes at a slower rate (Fig. 15).

For recordings of afferent activity peripheral nerves were cut from the cord centrally; for recording efferent activity nerves were detached from the body wall distally. Bursts of impulses in both modes could be correlated with passage of a peristaltic wave (Fig. 16).

The giant fibre system is not activated during normal peristalsis. The connexions of the giant fibre appear to be such as to mediate abrupt symmetrical overall with-

Fig. 15. Effects on the proboscis of electrical stimulation of the nerve cord. A stimulus of 4 V. was applied at a frequency of 5 per sec. for a duration of 50 msec. The length of time the stimulation was applied is indicated by the raised portion of the second trace.
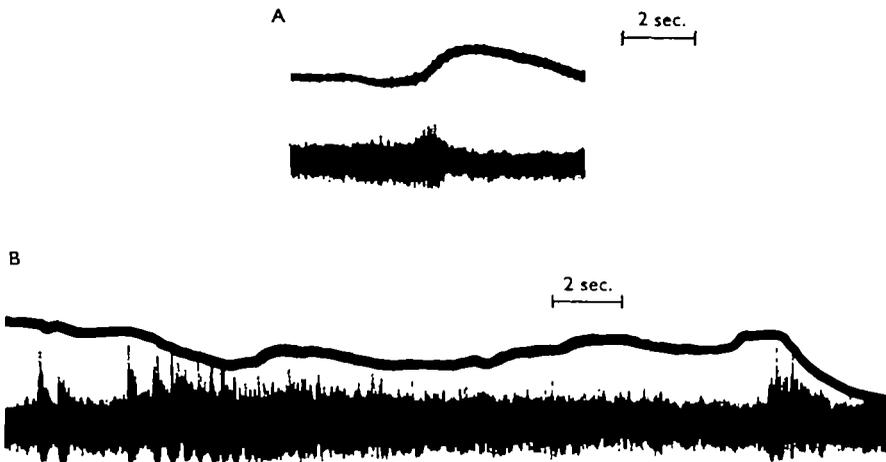
Fig. 16. A. Afferent activity in a peripheral nerve (lower trace) during contraction of the body wall in the same segment (upper trace). Upward deflexion indicates contraction. B. Efferent activity in a peripheral nerve during contraction of the body-wall in the same segment in the course of a conducted wave of peristalsis. A downward deflexion of the upper trace indicates contraction.

drawal responses to mechanical stimuli. Bursts of spikes are elicited by tactile stimuli applied to the body-wall and proboscis and propagate along the cord at 1·5 m./sec. (Fig. 17). The giant fibre does not appear to extend into roots, but afferent and efferent activity in the peripheral nerves can be correlated with activity in the giant fibre. The worm's response to firing of the giant fibre is a rapid anterior–posterior shortening, presumably effected by contractions of the longitudinal musculature. Direct stimulation of the cord posterior to the bifurcation of the circumpharyngeal connectives can drive the fast-conducting system directly, and its response will follow one-to-one up to

90 per second. Stimulation of the circumpharyngeal connective and peripheral nerves will fire the giant fibre synaptically, but such stimuli become ineffectual at frequencies greater than four per second. The giant-fibre system is non-polarized; it conducts at the same rate in the anterior and posterior directions.
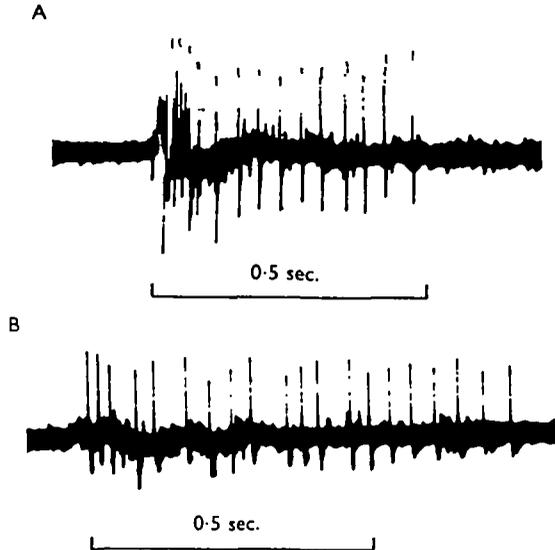

Fig. 17. Presumed activity in the giant fibre recorded from cord of *Urechis* when a tactile stimulus was applied to (A) the proboscis, and (B) the body wall in the region of mid trunk.

DISCUSSION AND CONCLUSIONS

The nerve cord of *Urechis* appears to contain a hierarchy of pacemaking elements, the lower members of which are normally held in check by the proboscis, which generates the descending waves of peristalsis. The evidence suggesting that the anterior pacemaker is located in the circumpharyngeal connectives does not exclude the possibility that a portion of the pacemaker system could be located peripheral to the connective. That the anterior pacemaker can be reset by interpolated electrical stimuli and is sensitive to low concentrations of acetylcholine indicates at least a neurogenic component in the pacemaker system. Acetylcholine appears to influence the behaviour of a proboscis preparation by increasing the amplitude of peristaltic waves recorded kymographically, by increasing the frequency of peristalsis and by producing a tonic contracture of the musculature of the body wall upon which peristalsis is superimposed. Higher concentrations of the drug may affect the muscular system directly; lower concentrations may be acting primarily on the nervous elements. The profuse secretions of the proboscis glands observed in preparations subjected to ACh appear similar to mucus secretions diagnostic of the muscarinic effects of ACh on mammalian systems, and could be indicative of cholinergic secretomotor junctions in *Urechis*.

Previous work on the peristalsis of earthworms (summary in Bullock & Horridge, 1965) suggests that peripheral reflexes enhance and adjust the apparently neurogenic

rhythm of peristalsis. In *Urechis* interaction of the nerve cord and body-wall appears necessary for conduction which depends upon an intact nerve cord, an intact body-wall and intact peripheral nerves between the initiation and termination points for a peristaltic wave. The cord presumably contains the major portion of the conducting and/or pacemaker elements, and its presence is necessary for the spontaneous generation of a propagated pulse. In earthworms lengths of body-wall free of nerve cord show no spontaneous activity and are incapable of conducting waves of peristalsis if inhibited by passive stretch; and it has been concluded that muscle-to-muscle conduction is of little significance (Prosser, 1934). In *Urechis* muscle-to-muscle conduction is not likely, although stretch is often capable of producing propagated contractions in pieces of nerve cord and body-wall too small to show autogenic rhythm, possibly by reflex excitation of conductile elements in the cord.

The nerve cord of *Urechis* contains two separate systems which conduct impulses along the length of the trunk in both directions. A slow, multisynaptic conduction system, which mediates the peristaltic waves, is presumably localized in the central neuropile of the cord and contains a hierarchical array of pacemaker elements. Each element in the hierarchy may function in the conduction of propagated waves, presumably by being reset by afferent signals from the body-wall which arise as a result of efferent activity propagated along peripheral nerves. Any interruption of the cord or the afferent/efferent loop to the body-wall tends to impede normal conduction of peristalsis and to release slower pacemaker elements from the checking influence of faster ones. The fact that a minimum mass of body-wall and nerve cord is necessary to give rise to a spontaneous, propagated rhythm may mean that such activity is produced by a fixed number of interconnected neurones.

The fast-conducting system, apparently consisting of a multicellular giant fibre functioning as a single unit, is not active during normal peristaltic movements, and may serve to mediate a startle response to tactile stimulation of the body-wall. This system, unlike the previous one, does not depend upon interaction of the cord and body-wall for conduction within it, although input to the system and output from it can occur all along the body, since stimuli applied to peripheral nerves can induce discharge, and efferent impulses correlated with activity of the giant fibre can be recorded from most peripheral nerves.

It is concluded that the pacemaker responsible for the anal breathing movements is located in the thin-walled muscular hind-gut, and that the pulse is conducted anteriorly by the nerve cord to a point where it is normally cancelled by a descending pulse from the proboscis. The pulse is presumably transmitted to the nerve cord by the peripheral nerves in the region of the anal bifurcation, and travels anteriorly by the same slowly conducting mechanism which is responsible for the descent of a pulse initiated at the proboscis.

Both the proboscis and hind-gut pacemakers respond with increased frequency of peristalsis when oxygenated sea water is used to replace nitrogenated sea water in these preparations. The behavioural responses, consisting of rapid irrigation of the burrow and hind-gut pulsations triggered by the sudden oxygen excess, may enable the previously quiescent animal to make efficient use of the oxygen and food resources of the incoming tide.

SUMMARY

1. This paper describes a neurophysiological investigation of the initiation and co-ordination of the peristaltic waves in *Urechis*.

2. Peristaltic waves are (*a*) initiated in the proboscis and propagated posteriorly, and (*b*) initiated in the hind gut and propagated anteriorly, provided that nerve cord, body-wall and peripheral nerves are intact.

3. There is a hierarchy of pacemaker regions in the nerve cord, effective over lengths as small as 30 mm. This hierarchy is normally dominated by the pacemaker in the proboscis.

4. The pacemakers respond with increased frequency to oxygen, epinephrine and acetylcholine in concentrations of 10^{-6}M. Eserine potentiates the acetylcholine response. The system is insensitive to 5-hydroxytryptamine.

5. The giant fibres conduct at 1·5 m./sec. and mediate a startle response; they are not involved in the conduction of the peristaltic wave.

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