Mar. Behav. Physiol. 1972, Vol. 1, pp. 1–27

Printed in Great Britain

The Control of Hindgut Motility in the Lobster, *Homarus gammarus* (L.)

1. Analysis of Hindgut Movements and Receptor Activity

W. WINLOW†‡ and M. S. LAVERACK

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

(*Received September* 1, 1971)

1. The sixth abdominal ganglion (6 A.G.) of the lobster *Homarus gammarus* (L.) innervates the rectum via the paired posterior intestinal nerves (P.I.N.'s) and the paired anal nerves. The anterior branches of the P.I.N.'s supply the anterior hindgut, the main faecal expulsion region, whilst the posterior branches (P.I.N.p.'s) supply the posterior region and the 5 extrinsic radial muscle groups around the anus.

2. Stimulation of the ventral nerve cord (V.N.C.) or the oesophageal connectives initiates co-ordinated hindgut movements, the defaecatory response. The nervous activity eliciting these movements passes down the P.I.N.'s. The anal nerves are devoid of motor function with respect to the hindgut.

3. In addition to neurogenic movements the rectum also undergoes non-coordinated, low amplitude longitudinal and circular muscle contractions. These are thought to be due to independently acting endogenous oscillators within the muscles themselves. The radial muscles of the anus also exhibit rhythmical contractions after an initial maximal contraction following stimulation of the P.I.N.p.'s.

4. Receptors responding to anal dilation and closure have been shown to exist in the anal nerves. They are non-specific soft cuticle receptors which are, apparently, positionally sensitive. These receptors are not thought to modulate motor output from the 6 A.G. to the rectum at the level of the 6 A.G.

5. Bifurcating motor axons are thought to exist in the P.I.N.'s.

6. It is concluded that the defaecatory response of the lobster is a centrally programmed phenomenon.

INTRODUCTION

The gut of reptantian decapods is innervated from two separate sources. Anteriorly there is the stomatogastric system (Allen, 1894; Dando and

†*Present address:* Department of Zoology, University of Glasgow, Glasgow, W.2, Scotland.

‡Supported by an S.R.C. studentship for which we express our thanks.

Laverack, 1969; Maynard, 1966; Orlov, 1926) which supplies the oesophagus, stomach and, perhaps, the midgut. Posteriorly the hindgut is supplied from the sixth abdominal ganglion (6 A.G.) (Alexandrowicz, 1909; Krohn, 1834; Lemoine, 1868; Police, 1908, etc.). Several authors have described the structure of both the 6 A.G. (Johansson and Schreiner, 1965; Krieger, 1880; Retzius, 1890) and the hindgut (Alexandrowicz, 1909; Janisch, 1923; Miller, 1910; Yonge, 1924).

Both Alexandrowicz and Orlov describe a plexus lying on the hindgut, but they are in dispute about its form. Alexandrowicz states that motor fibres running from the 6 A.G. are in direct contact with the axons of bipolar sensory cells innervating the hindgut. Orlov, whilst agreeing that sensory cells are present, denies that they make peripheral contact with the motor nerves. He also reports the presence of a further group of cells, the pyloric sensory cells, which innervate the hindgut. Their cell bodies lie on the pylorus, whilst their axons pass to the commissural ganglion and their dendrites ramify diffusely in the connective tissue of the midgut and the hindgut. The presence of receptors lying in the hypodermis underlying the soft cuticle of the anal lips and responding to anal dilation and closure has recently been reported (Winlow and Laverack, 1970). Thus it is obvious that there is a rich motor and sensory innervation of the hindgut.

It is our supposition that the major co-ordinating activities of the hindgut of reptantian decapods occur in the central nervous system (see also Campbell and Burnstock, 1968). The foregut is certainly under central control via a peripheral ganglion (Maynard, 1966; Morris and Maynard, 1970) and Miller (1910) has demonstrated that co-ordinated hindgut and anal movements can be produced in both *Homarus* and *Cambarus* by stimulation of the ventral nerve cord (V.N.C.).

Our aim in this present investigation is to elucidate some of the nervous pathways that influence hindgut motility in the lobster *Homarus gammarus* (L.).

MATERIALS AND METHODS

Anatomy

Both the gross anatomy of the posterior rectum and extrinsic radial musculature and the histology of the hindgut were determined using specimens fixed in alcoholic Bouin's fixative. The gross anatomical studies were carried out on preparations of the last two abdominal segments and the telson, which were dissected in 70% alcohol with the aid of a stereomicroscope. Serial sections of the rectum were prepared and stained using either Azan or Mallory's triple stain (Pantin, 1964).

The innervation of the rectum was investigated using methylene blue staining techniques.

Photomicrographs of the radial, longitudinal and circular muscles were prepared using the Nomarski interference-contrast apparatus on a Zeiss Standard GFL microscope. From such micrographs the sarcomere lengths of the various muscle groups were determined.

Physiology

In experiments to determine the precise nature of the movements of the hindgut, the last two or three abdominal segments were severed from the animals. The uropods were removed and the preparation pinned, ventral surface uppermost, in a wax-bottomed perspex experimental dish which was cooled by tap-water running through an outer water-jacket. This maintained the preparation, which was bathed in clean sea-water or *Homarus* saline (Pantin, 1964), at about 12°C.

The ventral integument was removed to expose the nerve cord, which was then freed from its connections with the somatic musculature. The somatic muscle groups lying dorsal to the V.N.C. and ventral to the hindgut were then removed leaving the 6 A.G./rectum complex intact, except for radial muscle group R1 (see Figure 1), which was invariably damaged during dissection.

The movements of the rectum and anus were monitored by means of two R.C.A. 5734 transducer valves to the anodes of which long balsa-wood wands were attached. The distal ends of the wands, which bore blunted steel insect pins, were placed at various points along the rectum and on the anal lips. The V.N.C. was stimulated by means of rectangular d.c. pulses from a Tektronix 161 pulse generator triggered by a 162 waveform generator. Stimuli were applied through platinum wire electrodes via a stimulus isolation unit. The results were displayed on either a Tektronix 561A or 565 oscilloscope, with either 3A74 or 3A3 preamplifier units, and were photographed using a Cossor oscilloscope camera and Ilford NS6 recording paper.

A second series of experiments was carried out in which the oesophageal connectives of an almost intact animal were stimulated. In this case sea-water was passed through a perspex dish in which the animal was secured ventral surface uppermost. The chelae and all the mouthparts and appendages were then removed and the connectives exposed by cutting through the ventral soft cuticle around the mouth. Posteriorly the hindgut was exposed in the usual manner leaving the nerves intact. Stimuli were delivered to the connectives in the way described above whilst the movements of the anus and the rectum were observed.

A third series of experiments involved the measurement of tension changes

in the radial muscles around the anus during stimulation. An isometric tension measuring device was set up using a transducer valve (Atwood *et al.*, 1965). A short pair of forceps was attached to the movable anode of the valve via a perspex coupling. A heat sink was found to be unnecessary and the valve was mounted in a perspex tube. In most of the experiments the connective tissue at the distal end of each group of radial muscles was held in the transducer forceps whilst a set of fixed forceps mounted on a Prior micromanipulator held the proximal end immobile. It proved possible to measure tension in the R4 muscle groups (see Figure 1) using only the transducer forceps attached to the anal lips and leaving the distal insertion of the muscle intact. Stimulation was delivered using suction electrodes.

The spontaneous activity of the hindgut was monitored in a fourth series of experiments. The rectal movements were recorded using a Devices single channel pen-recorder fed from the vertical signal output of the 565 oscilloscope using a 3A3 amplifier unit. In these experiments only one centimetre of midgut remained attached to the hindgut after dissection. As a preliminary to recording spontaneous activity the V.N.C. was always crushed at the abdominal 3–4 connectives in order to check that the hindgut gave the normal evoked response.

In experiments on the receptor activity of the hindgut only the last two abdominal segments were utilised. Initial experiments on the hindgut and anal soft cuticle were carried out by touching these regions with a camel-hair brush whilst recording from the appropriate nerves with platinum wire hook electrodes. The results shown in Figure 10 were produced using suction electrodes for both stimulation and recording from the P.I.N.'s of an isolated 6 A.G. Further attempts were also made to record receptor input from the P.I.N.p.'s. In these experiments the hindgut was artificially distended. This was achieved by injecting air into the hindgut down a fine polythene tube inserted anteriorly and tied in. The rectum was ligatured posteriorly using nylon thread.

Several methods of stimulating receptors around the anal lips were tried. In many cases one lip of the anus was passively deflected using a set of forceps attached distally to a Prior micromanipulator. The micromanipulator was fitted with a potentiometer which monitored its horizontal movements (see Shelton and Laverack, 1968). In other experiments the forceps were attached to a Southern Instruments type 940A pen unit driven at low frequencies by a Servomex, type LF51 (Mark 2), wave-form generator.

In all recordings the anal nerves were first cut centrally and then teased into fine bundles. In this way clear records of receptor responses in the anal nerves were made possible.

RESULTS

Anatomy

A. Innervation of the rectum by the sixth abdominal ganglion

The 6 A.G. lies in the midline directly dorsal to the superficial ventral muscles of the sixth abdominal segment. It bears six paired nerve roots and innervates the hindgut, which it underlies, via the paired posterior intestinal nerves (P.I.N.'s) and the paired anal nerves. The relationship of the 6 A.G. to the rectum is summarised in Figure 1.

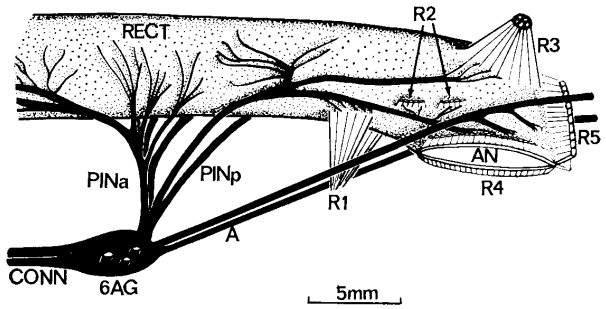

FIGURE 1 The isolated 6 A.G./rectum complex.

A. – anal nerves.
6 A.G. – sixth abdominal ganglion.
AN. – anus.
CONN. – 5–6 connectives.
P.I.N.a.'s – anterior branches of posterior intestinal nerves. These branches supply the anterior and middle regions of the hindgut and also run onto the midgut.
P.I.N.p.'s – posterior branches of the posterior intestinal nerves, which supply the posterior hindgut and the extrinsic radial muscles of the anus.
R1 – paired antero-ventral radial muscles.
R2 – paired lateral radial muscles.
R3 – paired dorsal radial muscles.
R4 – perianal radial muscles.
R5 – paired posterior oblique radial muscles.
RECT. – rectum.

The P.I.N.'s divide typically into anterior and posterior branches. The anterior branches of the P.I.N.'s (i.e. the P.I.N.a.'s) supply the anterior region of the rectum – the main faecal expulsion region (see below) – and also pass onto the midgut, whilst the posterior branches (i.e. the P.I.N.p.'s) supply the posterior region of the rectum and the extrinsic muscles of the anus as shown in Figure 1. The P.I.N.p.'s are generally much finer than the P.I.N.a.'s

which also give off a series of side-branches to the mid region of the hindgut. The positions of these side-branches are rather variable as are the positions of the P.I.N.p.'s which occasionally arise from the anal nerves. All these nerves eventually come to lie below the thin tough investing connective tissue layer of the hindgut. Their size decreases as they pass distally, due to the ramifications of their axons over the gut musculature. Methylene blue staining indicates that several axons in the P.I.N.'s bifurcate many times and give off branches – dividing in the same pattern as the main nerve trunks – to supply anterior, posterior and middle regions of the rectum. If the 6 A.G. is desheathed dorsally and methylene blue stain allowed to run back along the axons, during progressive dissection, it can be seen that axons running to supply the P.I.N.'s on either side often bifurcate and thus send a major branch to both P.I.N.'s. Whether single axons divide and send branches to all the divisions of both ipsilateral and contralateral P.I.N.'s has not been established.

The anal nerves, in the main, carry afferent information from the sensory structures of the telson, but they also carry some motor fibres which supply the telson flexor muscles. Further, they give off small branches medially which terminate in the region of the anus.

B. The structure of the hindgut

The anatomy of the rectum of *Homarus* is, with minor variations, similar to that of *Nephrops* (Yonge, 1924). Anteriorly, at the midgut/hindgut junction, there is a glandular swelling due to the presence of the tegumental glands which lie external to the muscles, but within the tough connective tissue sheath of the rectum.

The musculature of the rectum is arranged as an outer coat of circular muscle within which lie the separate strands of longitudinal muscle, commonly six in number. These strands of longitudinal muscle lie within longitudinal ridges that project into the lumen of the hindgut. All the intestinal muscles are striated. The lumen of both the hindgut and the foregut is lined with a layer of thin cuticle. The anterior region of the hindgut is relatively much more muscular than either the adjacent posterior midgut or the posterior hindgut. The lumen of the anterior hindgut is of much greater diameter than that of the posterior hindgut. Posteriorly the floor of the hindgut is open to form the anus. Externally the anus appears as a longitudinal slit in the ventral soft cuticle of the telson. At the sides of this slit the cuticle is invaginated to form a pair of sulci. The circular muscles of the rectum continue in the anal region as a layer of arched muscle fibres. Five groups of extrinsic radial muscle fibres around the anus take their proximal insertions from the proctodaeal cuticle. Their distal insertions are as follows (see Figure 1):

Muscle group R1 – the paired antero-ventral radial muscles – insert into the ventral soft cuticle posterior to the last adbominal sternite.

R2 and R3 – the paired lateral and the paired dorsal radial muscles – insert into the lateral and dorsal walls of the telson respectively.

R4 – the perianal radial muscles – insert onto the islands of hard cuticle lying lateral to the anus and onto which the misnamed (Schmidt, 1915) anal compressor and anal dilator muscles, which flex the telson, also insert.

R5 – the paired posterior oblique radial muscles are rather ill-defined in origin. They insert mainly into the dorsal wall of the telson a few millimetres behind the anus.

There is no anal sphincter muscle.

The sarcomere lengths of the various muscle groups have been measured and are summarised in Table I.

TABLE I

Muscle group	Sarcomere length (μ)
R1	6–7.5
R2	6–8
R3	5–7.5
R4	7–8
R5	7
Circular muscles of rectum	7
Longitudinal muscles of rectum	5–6

Physiology

Our initial work was to repeat the experiments carried out by Miller in 1910. Our results agree with his in several respects (Table II) but there are two major discrepancies. The first is that we find the anal nerves to be devoid of any motor function with respect to the radial muscles of the anus. Miller found that stimulation or severance of the anal nerves was sufficient to produce a single anal opening; we do not. A second point of disagreement is with Miller's assertion that direct stimulation of the hindgut "causes the passage of the usual contraction wave followed by anal opening"; in our experiments this caused only localised hindgut movements.

A. Determination of the form of the hindgut movements

To determine the manner of movement of the hindgut the transducer wands were placed at various points along its length and on the lips of the anus. The distances across which their tips were deflected were determined by direct measurements. Simultaneous recordings of these movements were made and their sequential nature worked out. Figure 2 shows an oscilloscope trace typical of those obtained by this method indicating the response of the hindgut to stimulation of the V.N.C. In this case transducers were placed on the right

TABLE II

Experiment	Results	
	Miller	Present work
1. Stimulate V.N.C.	Hindgut peristalsis accompanied by rhythmic anal movements	
2. Stimulate V.N.C. with anal nerves severed	As above	
3. Stimulate V.N.C. with P.I.N.'s severed, but anal nerves intact	Single anal opening	No movements
4. Sever anal nerves	As above	
5. Stimulate anal nerves directly	Anal opening on both sides. This is most prominent on the stimulated side	No movements
6. Stimulate P.I.N.'s directly	—	Normal hindgut movements
7. Stimulate hindgut directly	Co-ordinated movements of rectum and anus	Only localised hindgut movements

Miller in his experiments utilised the lobster *Homarus*† and the crayfish *Cambarus*† and worked on whole animal preparations. He stimulated the animal for between 3 and 20 seconds using the "interrupted current of an inductorium". Present report is for the lobster *Homarus gammarus* (L.) and involved only the last two or three abdominal segments. We applied 5–10 seconds of stimulation at 20–40 Hz using 1–3 ms rectangular pulses.

†No further information given as to species.

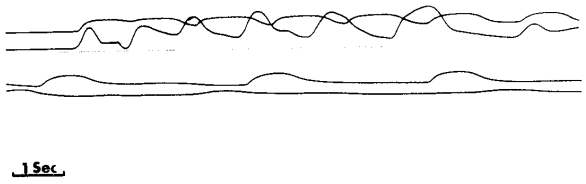

FIGURE 2 Transducer record of the movements of the hindgut occurring as a result of stimulation of the V.N.C. of a fresh preparation. Continuous trace.

First beam – transducer on right lip of anus – upward deflection denotes anal opening. Second beam – transducer at midgut/hindgut junction – upward deflection denotes posteriorward motion due to contraction of longitudinal muscles, whilst downward deflection denotes anteriorward motion due to circular muscle contraction. Third beam – stimulus marker – 40 Hz at a pulse width of 3 ms.

lip of the anus (upper beam) and immediately posterior to the midgut/hindgut junction (lower beam). The responses last for variable lengths of time from animal to animal and vary with the condition of the preparation. In the initial stages the anus is held widely open, especially in fresh preparations, and exhibits only minimal closing contractions. Hindgut movements during this period are due to bursts of activity in the P.I.N.'s (see Winlow and Laverack, 1972). Subsequently the hindgut exhibits co-ordinated activity, whose onset is marked by a series of rhythmic peristaltic waves passing back down the rectum (see Figure 3). Accompanying the peristaltic waves is the anal rhythm. As the anus closes, the anterior rectum, which is very muscular, contracts longitudinally in a localised manner resulting in a net rearwards movement of the midgut/hindgut junction (Figure 3(b)). This longitudinal muscle contraction is then followed by a powerful circular muscle contraction giving rise to a posterior-going peristaltic wave. This wave is initiated in the region of the midgut/hindgut junction (see Figure 3(c)). During this time the anus remains fully closed, but it opens again as the wave passes posteriorly (Figure 3(d)). The anus is then kept fully open for a short time (Figure 3(e)) and the next wave of contraction then follows. During the passage of the peristaltic wave the hindgut may elongate anteriorly by as much as 3 mm. The middle region of the hindgut exhibits very little movement during this cycle and is only weakly contractile, as emphasised by the paucity of musculature in this region. After stimulation the hindgut movements continue for a short time, but often in a less well co-ordinated manner, as is demonstrated in Figure 2. Thus defaecation is accomplished by contraction of the faecal expulsion region, E, (Figure 3) of the rectum acting in synchrony with the extrinsic radial muscles which open the anus thus allowing the faeces to pass to the exterior. Anal closure may be a function of the arched muscle fibres or may be due to elastic rebound of the ventral soft cuticle around the anus.

B. Control of the hindgut movements

In a series of experiments on the virtually intact animal it was found that stimulation of either oesophageal connective, both anterior and posterior to the commissural ganglion, would produce the response of the hindgut described above.

A further understanding of the hindgut co-ordinating mechanism can be gained by sectioning both the rectum and the nerves supplying it.

1. *Sectioning of the hindgut*

In experiments in which the hindgut was sectioned at a position between the P.I.N.a.'s and the P.I.N.p.'s, leaving the nerves intact, the co-ordinated nature of the hindgut response was unaffected (Figure 4). The form of the contraction did, however, change probably due to interference with the

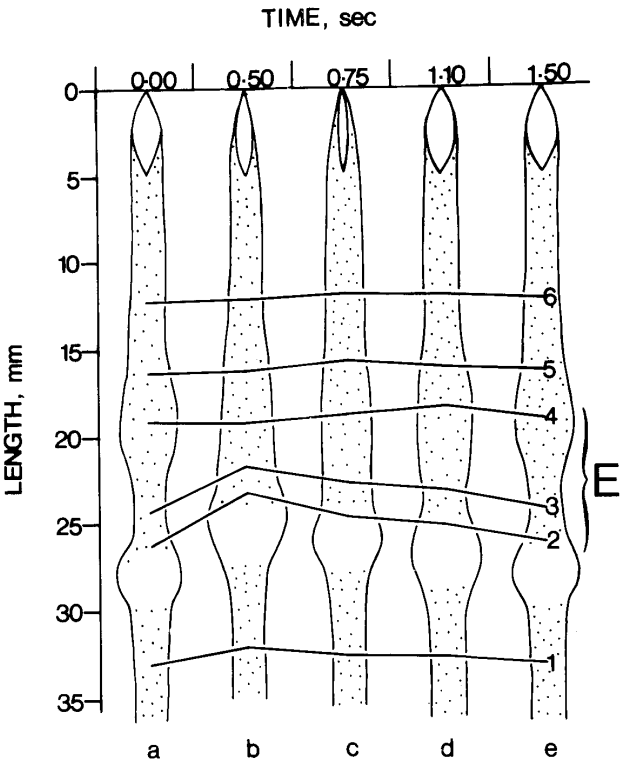

FIGURE 3 Motility of the hindgut during stimulation of the V.N.C. One cycle of th secondary phase of the response is represented.

The figure is drawn to scale and represents the movements of a series of points (1 to 6 along the hindgut. Anal movements are also shown. These movements were recorded usin R.C.A. 5734 transducers as in Figure 2, from which some of the data were taken. Measure ments of the amount of movement of the transducer wands were taken directly using a scal marker laid in the bath alongside the preparation. The timing of the various sections of th response is shown on a non-linear scale at the top of the diagram. Each cycle of movemen takes 1.5 sec.

Point 1 moves passively following the movements of 'E', the main faecal expulsion regio of the rectum, in a somewhat damped manner. Points 5 and 6 move weakly, but the peristalti wave does pass.

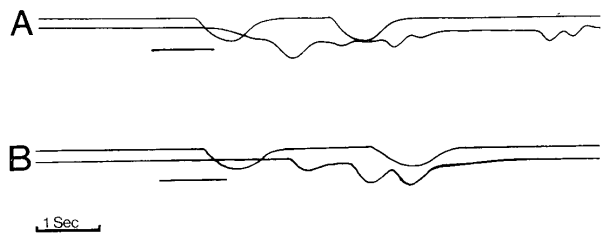

FIGURE 4 The effect of sectioning the rectum on the co-ordination of the rectal and anal responses after stimulation of the V.N.C. (40 Hz, 3 ms pulses).

Upper beam – transducer on left lip of anus – downward deflection denotes anal opening. Lower beam – transducer at midgut/hindgut junction – upward deflection denotes posteriorward movement and downward deflection denotes anteriorward movement. The horizontal bars are the stimulus markers.

(A) intact preparation.

(B) hindgut sectioned between P.I.N.a.'s and P.I.N.p.'s. The innervation remains intact.

longitudinal muscle groups. As long as the innervation remained intact several cuts across the alimentary canal could be made without affecting the basic co-ordination of the response.

2. *Sectioning the nerves*

a) *Anal nerves* Cutting the anal nerves does not disturb either the frequency of the anal rhythm or the movements of the anterior region of the rectum. The co-ordinated nature of the response is also unaffected.

b) *P.I.N.a.'s* Figure 5 shows the effects of gradual elimination of the anterior rectal nerve supply. Cutting the main trunks of the P.I.N.a.'s, whilst leaving the side-branches intact, prevents the circular muscle contraction wave and greatly reduces the longitudinal muscle contraction (Figure 5(C)). Severing the side branches of both P.I.N.a.'s then virtually abolishes the response of the anterior region of the hingut to stimulation of the V.N.C. (Figure 5(D)). Sectioning of a single main trunk whilst leaving all its side branches intact reduces considerably the circular muscle contraction and alters the phase of the longitudinal muscle contraction (Figure 5(B)). During all these experiments the response of the anus remained totally unaffected.

c) *P.I.N.p.'s* Direct and simultaneous stimulation of both P.I.N.p.'s causes anal opening (Figure 6(A)). The response of the right anal lip may be abolished by sectioning the right P.I.N.p. peripheral to the stimulating electrodes (Figure 6(B)). Prolonged bursts of stimuli to the P.I.N.p.'s will cause the anal rhythm (Figure 7(F) and (G)).

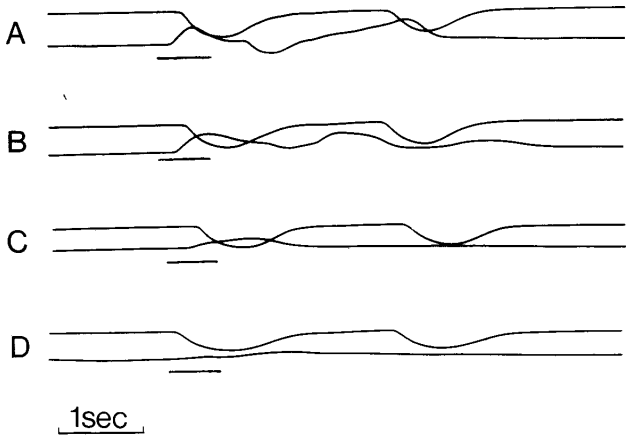

FIGURE 5 The effect of progressive extirpation of the P.I.N.a.'s and their side-branches on the movements of the hindgut when the V.N.C. is stimulated (40 Hz, 3 ms pulses). Horizontal bars are stimulus markers. Transducer placements as in Figure 4.

(A) system intact. Normal response.

(B) main trunk of right P.I.N.a. sectioned peripherally leaving side branches intact.

(C) main trunk of left P.I.N.a. sectioned peripherally leaving side branches intact.

(D) side branches of both P.I.N.a.'s sectioned.

For further details see text.

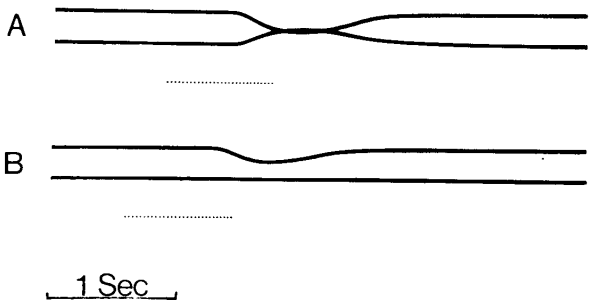

FIGURE 6 Simultaneous stimulation of both P.I.N.p.'s and monitoring of the movements of both anal lips. Upper beam – left anal lip. Lower beam – right anal lip.

(A) both P.I.N.p.'s intact.

(B) right P.I.N.p. several peripheral to the stimulating electrodes.

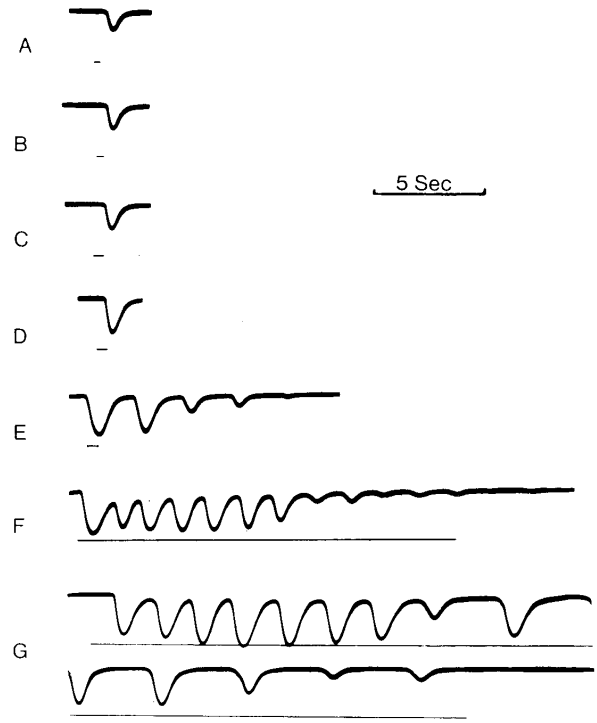

FIGURE 7 Movements of left anal lip in response to bursts of stimuli at constant frequency (20 Hz) but of gradually increasing duration. Stimuli were delivered down the intact P.I.N.p.'s. The numbers of pulses per burst were as follows:

(A) 10; (B) 13; (C) 15; (D) 18; (E) 20; (F) 180; (G) 410.

Figures (F) and (G) show the effects of prolonged periods of stimulation. The decrease in contraction amplitude towards the end of stimulation may be due to fatigue.

For further details see text and Figure 8.

3. *The anal rhythm*

The anal rhythm is one facet of the highly co-ordinated movements of the hindgut. In fresh preparations it occurs during the secondary phase of evoked rectal movements. It can also be elicited by stimulation of the P.I.N.p.'s and there is no change in its form after these nerves are cut centrally. Increasing the duration of constant frequency stimulation delivered to the P.I.N.p.s' causes the radial muscles to contract monotonically as the number of pulses

delivered increases (Figure 7(A)–(D)). A maximal contraction is reached (Figure 7(D)) after which any increase in the number of pulses delivered causes further oscillatory contractions of the radial muscles (i.e. the onset of the anal rhythm) Figure 7(E)).

In an attempt to determine the underlying mechanisms of the anal rhythm further experiments were carried out. In these the radial muscle groups were individually attached to the transducer and tension changes in them during stimulation of the P.I.N.p.'s were recorded. Figure 8 shows a trace in which

FIGURE 8 Tension response of immobilised right anal lip (i.e. R4 radial muscle group) due to stimulation of cut peripheral ends of P.I.N.p.'s using a suction electrode. Stimulus characteristics: 25 Hz, 0.1 ms pulses. Horizontal bar is stimulus marker.

tension changes in the perianal radial musculature (R4) were monitored during stimulation of the P.I.N.p.'s. The trace obtained is very similar to those recording movements of the anal lips (see upper trace of Figure 2). There is an initial delay between stimulus and response of about 0.5 sec. This is followed by a sustained contraction (with ripples of anal rhythm) whilst stimulation is maintained. After the stimulus ceases a further contraction (or in many cases a series of contractions) occurs. This inherent rhythmicity was found to occur in all five radial muscle groups.

C. Spontaneous motility of the hindgut

It was found that the hindgut could show many forms of spontaneous activity. Such spontaneous motility was not dependent on the presence of the 6 A.G. which seemed to exert little or no influence on the rectum when not actually driving it. The most typical movements were due to relatively slow circular muscle contractions often associated with rather faster longitudinal muscle contractions, at least in fresh preparations (see Figure 9(A)). Later in the experiments these movements often became desynchronised. Longitudinal and circular muscles were then found to beat at their own rate and to exhibit the phenomenon of phase drift (Figure 9(B)). In addition, the longitudinal muscle strips were also found to beat independently of one another, pulling the cut end of the midgut either to the right or left during contractions. In many cases only longitudinal muscle strips of the right or left side would contract, the other side remaining inactive. Many different rhythms were seen to develop, often slowly increasing and decreasing in frequency (Figure 9(B)).

The maximal excursion made by any given point in the region of the

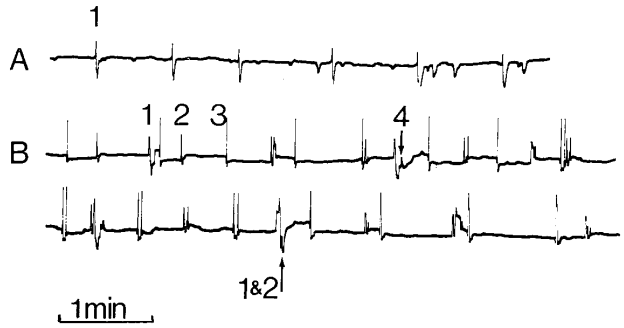

FIGURE 9 Spontaneous hindgut contractions recorded directly posterior to the midgut/hindgut junction using an R.C.A. 5734 transducer diode. In this case the 6 A.G. was intact. Upward deflection denotes posteriorward movement. Downward deflection denotes anteriorward movement.

(A) longitudinal muscle contractions directly preceding circular muscle contractions during the first 10 minutes of the experiment. The circular muscle contractions become more complex at the right.

(B) continuous trace starting 50 minutes after initiation of the experiment.

1 – rhythmic longitudinal and circular muscle contractions as in (A).

2 – longitudinal muscle contractions pulling the cut end of the midgut to the left. These become double contractions before returning to single contractions.

3 – longitudinal muscle contractions to the right. These also double up.

4 – longitudinal muscle contraction to the right.

1&2 – a fusion of 1 and 2. Contractions gradually slowed until 15 minutes after the end of the trace, when all activity ceased. Crushing the V.N.C. still elicited normal hindgut movements.

midgut/hindgut junction was rarely more than 0.5–1 mm, which is a good deal less than the movements made during evoked activity (up to 3 mm – see Figure 3). The movements of the anterior hindgut were never observed to be co-ordinated with movements of the anus (which rarely opened spontaneously during these experiments) although preliminary and post-experimental checks generally revealed the radial musculature to be in a completely functional state.

D. Proprioceptors on the hindgut

Under no circumstances has it proved possible to record receptor activity in the P.I.N.'s. Neither hindgut movements nor artificial distension of the hindgut elicited any recordable receptor activity. Stimulation of one P.I.N.a. will often produce an output in the contralateral P.I.N.a. as is shown in Figure 10. Three sizes of single spikes are displayed, the fastest having a

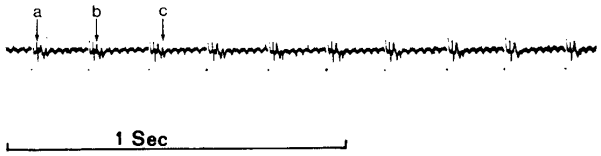

FIGURE 10 Response elicited in right P.I.N.a. by stimulation of the left P.I.N.a. at 6 H and with 1 msec pulses. Suction electrodes were utilised for both recording and stimulati purposes.

Three spikes (a, b and c) per stimulus pulse were elicited. The distance apart of the recordi and stimulating electrodes was 12.5 mm ± 2.5 mm and the conduction velocities of t spikes between stimulus and recording electrodes have been calculated. They are as follows:

a 5–7.5 metres/sec.

b 2.5–3.7 metres/sec.

c 2–3.5 metres/sec.

Dots indicate stimulus pulses.

conduction velocity of 5–7.5 metres/sec., whilst the slowest has a conductio velocity of only 2–3.5 metres/sec., but these are thought to be due to outpu from bifurcating motor fibres (see Discussion).

E. Anal proprioceptors

Although it has not been possible to demonstrate the presence of afferen from the hindgut, a number of fibres responding to movements of the anu itself have been found in the anal nerves (Winlow and Laverack, 1970).

1. *Anatomy* Methylene blue staining of the anal nerves in the region of th anus indicates that in at least one ventrally going branch on each side there one large bipolar sensory cell. The sense cell body lies deep alongside the anu and the dendrite passes posteroventrally to lie on a portion of the hypoderm below the soft cuticle of the anus (see Figure 11). The dendrite then loops t run anteriorly and finally ramifies in the hypodermis on the same side of th anus.

2. *Physiology* It was possible to elicit non-specific receptor responses in th anal nerves by touching the soft cuticle around the anus with a camel-hai brush.

More precise information was obtained by making recordings of th responses of the anal receptors in teased anal nerves whilst driving the radia muscles of the anus by stimulation of the P.I.N.p.'s. Figure 12 demonstrate the activity of receptors discharging during anal opening, at which time th soft cuticle surrounding the anus was deformed. Removal of the overlyin cuticle does not interfere with this response, but cutting the underlyin hypodermis abolishes it. The receptor cell described above is thought t

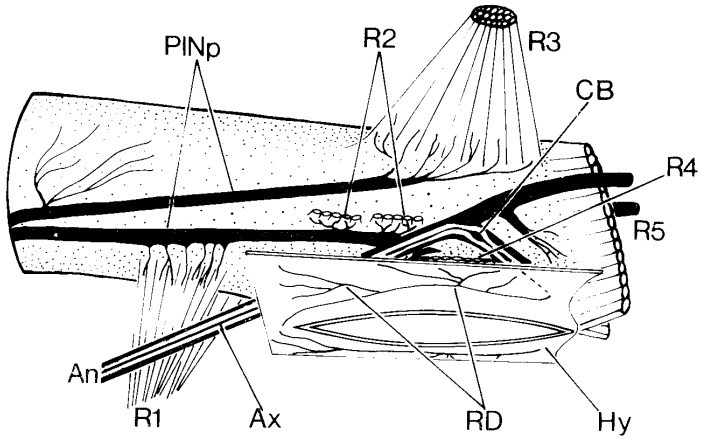

FIGURE 11 Diagrammatic representation of the position of the bipolar sensory cell postulated as responding to anal dilation.

R1 to R5 – radial muscle groups as shown in Figure 1.
An. – left anal nerve.
Ax. – axon of receptor cell.
C.B. – receptor cell body.
Hy. – hypodermis around anal orifice.
P.I.N.p. – posterior branches of posterior intestinal nerves.
R.D. – ramifying dendrites of receptor cell lying in the hypodermis.
The ventral soft cuticle is not represented.

FIGURE 12 Response of a soft cuticle receptor of the anal lip during anal opening.
Upper beam – Activity of the receptor in the teased left anal nerve, cut directly posterior to the 6 A.G.
Lower beam – Record of the movements of the left anal lip recorded with an R.C.A. 5734 transducer valve (downward deflection denotes anal opening).
(A) spontaneous activity of the receptor with the anus in the closed position. Mean frequency of output is approximately 3 Hz.
(B) activity of the receptor during anal opening elicited by simultaneous stimulation of both P.I.N.p.'s with 3 msec, pulses at 50 Hz for approximately 1.5 sec. (dotted line indicates stimulus pulses).

generate this response. In a number of preparations a second receptor respond ing to anal closure has also been found. This often occurs in the same teased bundle as the dilation receptor (Figure 13). The anatomical position of the propricoceptor responding to anal closure is unknown.

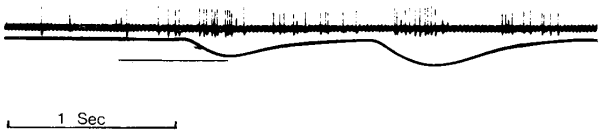

FIGURE 13 Opening and closing responses of receptors on the left anal lip.

Upper beam and lower beam as in Figure 13. Anal movements were produced by stimula tion of the V.N.C. (40 Hz and 3 msec. pulses).

Note the increased amplitude of movement in the second cycle, producing greater separa tion of the responses in time. The dilation receptor responds over most of the range of anal opening, whilst the closing receptor responds only when the anal orifice is almost closed.

Horiztontal bar denotes stimulus pulses.

Passive opening of the anal lip revealed the dilation receptor to be phaso tonic. In Figure 14(A) the frequency of the response is shown to be dependent on the extent of anal opening. During rapid cyclical movements of the anal lip as in Figure 14(B), only the phasic portion of the response can be recorded due to the short time over which the stimulus is applied. Movement of the anal lip close to its maximum degree of opening (Figure 15(A)) gives a typical, slowly

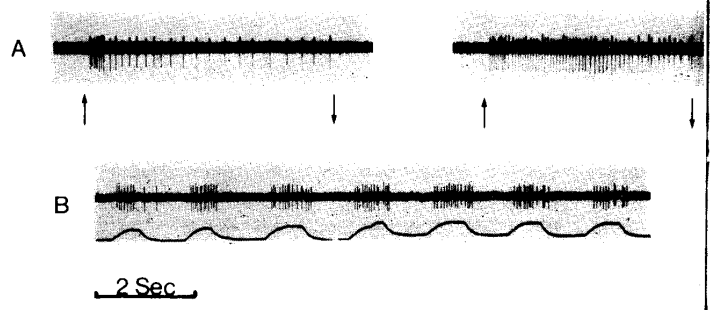

FIGURE 14 Passive movements of the anal lips producing responses of the openi receptor. In all cases the right anal lip was moved by a pair of forceps mounted on a Pri micromanipulator.

(A) arrows pointing upwards denote the onset of the stimulus whilst those pointi downwards denote its cessation. In both cases there is a phaso-tonic response. In th second case the anus was opened more widely, thus causing greater hypoderm deformation and giving rise to a higher frequency of both phasic and tonic outp from the receptor.

(B) rapid cyclical movements of the anal lip monitored with a transducer valve. Only t phasic component of the response was recorded, due to the rapidity of the movement

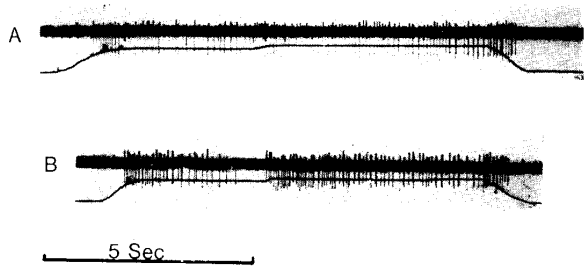

FIGURE 15 Responses of the dilation receptor to imposed opening of the anal lip monitored by a potentiometer mounted on a Prior micromanipulator.

(A) the anus was initially opened to 95% of its natural maximum and was then opened wider to 110%.

(B) the initial opening was 80% of the natural maximum and this was later increased to 100%.

In both (A) and (B) the initial opening caused a phaso-tonic output and wider opening caused a similar response, but at a higher frequency. The final phasic burst as the anus was closed is thought to have been due to rebound of the forceps. Two units may have been involved in both (A) and (B).

adapting response, whose frequency can be further increased by additional opening of the anal lip. If the anal lip is only moved to 80% of its natural open position the response totally adapts, but moving the lip to the fully open position again increases the tonic frequency of the receptor output with a resultant increase in the adaptation time.

The evidence presented in Figure 15 suggests that both phasic and tonic components of the receptor response are positionally sensitive with respect to anal dilation. The receptor does not appear to be strongly velocity sensitive. In Figure 15 the receptor is only shown to respond strongly when the anus is passively opened almost to its natural maximally opened position. Figures 12 and 13, however, show that the response to active anal opening is initiated at the beginning of the active opening cycle. This apparent discrepancy may well be due to the fact that when the anal lip is passively moved, the forceps attached to it at one point tend to pull the parts of the anal lip, anterior and posterior to the attachment of the forceps, into a sharply angled shape. Thus the soft cuticle at the extreme anterior and posterior ends of the anal lip is unlikely to be deformed to quite the same extent as when active anal movements occur. In such cases contractions of the R4 radial muscles pull the anal lip open in a smooth arc, thus deforming the hypodermis and the receptor endings associated with it, in a different manner.

Further analysis of the output of the receptor responding to anal dilation was attempted using a variety of movements at different frequencies and

amplitudes. The results obtained proved inconclusive due to the imprecise nature of the response from cycle to cycle. Figure 16 shows that wider opening of the anus causes a higher degree of phasic discharge and above 70–80% of natural anal opening there is little increase in the discharge frequency of the phasic component of the receptor.

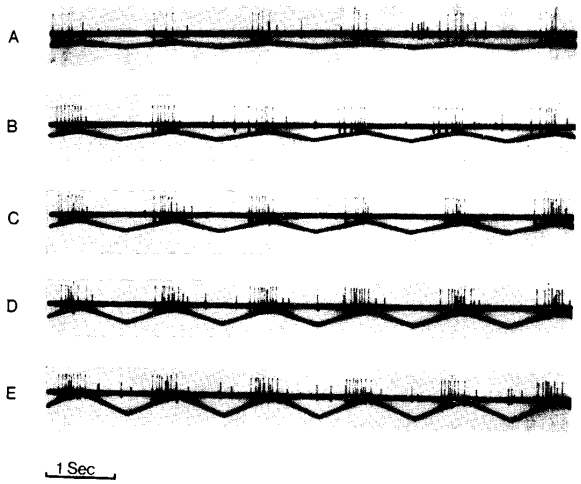

FIGURE 16 Triangular movements at constant frequency (0.7 Hz) imposed on the right anal lip and responses recorded from the right anal nerve. The movements were produced using a waveform generator to drive a pen arm on which the forceps, attached to the anal lip, were mounted. The amplitude of the movements was gradually increased.

Upper beam – activity recorded in the teased right anal nerve. Lower beam – monitor of the imposed waveform, upward deflection denotes anal opening.

(A) anal lip opened to 40% of its natural maximum.

(B) 70%; (C) 80%; (D) 120%; (E) 160%.

The frequency of the receptor discharge remains fairly constant above about 70% of natural anal opening.

In many cases more than one unit was found to discharge during anal closure or anal dilation. Figure 17(A) and (B) shows several units responding to active anal closure. In Figure 17(C) a two-unit response to passive anal opening is demonstrated. Thus it seems that a number of units, which discharge during anal movements, can be isolated from the anal nerves. No evidence of any complex form of stretch receptor has as yet been discovered. It is, therefore, assumed that these receptors are probably unspecialised mechanoreceptors associated with the hypodermis underlying the soft cuticle of the anal lips.

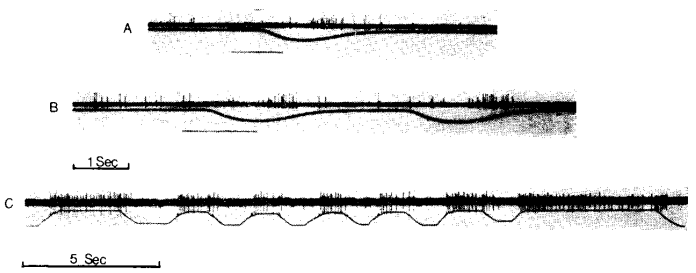

FIGURE 17 Several units responding to both anal opening and anal closure. In (A) and (B) the lower beam monitored active movements of the left anal lip as in Figure 13, whilst in (C) imposed movements of the left anal lip were monitored as in Figure 15. The movements in (A) and (B) were produced by stimulation of the V.N.C. at 40 Hz with 3 msec. pulses. Dots indicate stimulus pulses.

(A) and (B) three separate units responded to anal closure. The two traces demonstrate the variability of the response.

(C) two units were involved. They discharged phaso-tonically.

DISCUSSION

Anatomy

According to a number of authors (Krohn, 1834; Lemoine, 1868; Police, 1908; Alexandrowicz, 1909; Miller, 1910; Janisch, 1923; Yonge, 1924 and Orlov, 1926) the P.I.N.'s of *Astacus*, *Homarus*, *Palinurus*, *Cambarus* and *Nephrops* arise from a single median root which then divides sending a pair of branches anteriorly and a single median branch to the anus. Krohn states that the root of the P.I.N.'s in *Homarus* and *Astacus* may sometimes be paired. The usual situation in *Homarus gammarus* is that the P.I.N.'s arise from paired roots which then bifurcate to send ipsilateral branches anteriorly and posteriorly (Figure 1). As mentioned in the results these nerves are very variable in their finer details.

The term anal nerve (Keim, 1915) is something of a misnomer since the side branches of this nerve to the anus are very minor in comparison to the main branch which passes to the telson. These nerves should be certainly renamed perhaps as the "caudal" or "terminal" nerves.

Further misleading terminology occurs in the case of the anal dilator and anal compressor muscles (Schmidt, 1915), since these are not directly involved in the functioning of the anus. Larimer and Kennedy (1969) show that these muscles may *incidentally* change the shape of the anal orifice since they insert distally onto the soft cuticle around the anus. Their function is to flex the tail

fan by their action on the telson and Larimer and Kennedy suggest that they behave like the serial homologues of the tonic, superficial flexor and the phasic, anterior oblique muscles of more anterior abdominal segments.

The sarcomere lengths of the muscles associated with the hindgut all lie in the upper part of the 4–8 μ range (Table I). According to Hoyle (1967) this would imply passively or gradedly responding fibres. This fits with their properties of slow contraction and with the slow rise times and low amplitudes of their membrane responses as recorded by Ebara (1969).

Physiology

The discrepancies between our work and that of Miller (Table II) may have been due in part to his use of animals in which the anal nerves and P.I.N.p.'s were fused. His diagram of the 6 A.G./rectum complex of *Homarus* shows this fusion. Thus severance of the anal nerves proximal to their division from the P.I.N.p.'s could well have produced a single anal opening. However, it is difficult to see why stimulation of such combined nerves would produce only a single opening and not the anal rhythm. If we assume Miller stimulated the anal nerves distal to their division, current spread to the radial muscles may have been responsible for the contraction, especially as he states that the extent of anal dilation was more marked on the stimulated side. In addition his assertion that direct stimulation of the hindgut would produce co-ordinated movements was unsubstantiated. Perhaps this was again due to current spread.

From the results set out above it can be seen that although the hindgut is capable of spontaneous, unco-ordinated, rhythmic movements, the act of defaecation in *Homarus* is a centrally programmed phenomenon, whose basic pattern of co-ordination cannot be distributed by disruption of the hindgut musculature (Figure 4). Experiments in which the oesophageal connectives of an almost intact animal were stimulated, indicate that the "local" co-ordination system within the 6 A.G. is subservient to higher control mechanisms, probably located within the brain. The nature of the motor output to the hindgut has now been determined (Winlow and Laverack, 1972).

The independent rhythmicity of longitudinal and circular muscles (Figure 9) suggests that spontaneous hindgut motility is due to the presence of numerous independent oscillators, situated within the longitudinal and circular muscles. Such rhythmicity occurs whether the 6 A.G. is present or absent. Similar independent contractions of longitudinal muscle strands have been observed in the hindgut of other macrurous decapods (Alexandrowicz, 1909; Ebara, 1969). Ebara (working on the crayfish *Procambarus clarkii*) concluded that each individual muscle strip was governed by its own endogenous pacemaker located in the anterior region. However, obliteration of this region resulted in pacemaker activity being shifted more posteriorly with a subsequent decrease in frequency. Successive ablations of pacemaker sites caused a steady diminution in the contraction rate until contractions ceased. Ebara also demonstrated that the rate of depolarisation of the muscle membrane was very slow (from 60 to 190 mV per sec. in the rapid phase of depolarisation). Prosser, Nystrom and Nagai (1965) assert that the electrical activity of the crustacean abdominal intestine is nervously conducted. They applied either drugs or direct stimulus, however, and either of these would necessarily affect the motor nerve terminals or the nerves themselves. Thus their experiments do not exclude the possibility of a myogenic component. Our experiments indicate that both neurogenic and myogenic components exist.

The radial muscles of the anus rarely contract spontaneously although the anal rhythm may continue for long periods after cessation of stimulation of the P.I.N.'s (57 sec. in one of Miller's experiments). Perhaps the radial muscles may have similar embryological origins to those of the longitudinal muscles (Winlow, 1970). Their inherent rhythmical properties, following stimulation, may be produced by a mechanism similar to that causing spontaneous rhythmic longitudinal muscle contractions. Thus an oscillatory mechanism within them may be activated by the arrival of nervous impulses sufficient to produce a maximal muscle contraction (see Figure 7).

The delay of up to half a second between the delivery of stimulus pulses and the onset of the response of the hindgut muscles is unlikely to be explained by conduction delays in the nerves. However, it may be due to a very slow muscle depolarisation phase such as that observed by Ebara in *Procamabrus*. Intracellular recording would clarify the situation, but so far it has been impossible to insert microelectrodes into the radial muscles.

The receptors on the soft cuticle around the anal lips are reminiscent of the cutaneous mechanoreceptors described by Pabst and Kennedy (1967). The somata of these cells are located in the proximal region of the first and second ganglionic roots of each abdominal ganglion of the crayfish *Procambarus clarkii*. They innervate the soft cuticle associated with the flexor muscles and the bases of the swimmerets. Their input produces reflex suppression of motor discharge to the postural flexor muscles and swimmerets. The units we describe around the anal lips also respond to deformation of the hypodermis of the ventral soft cuticle and may be envisaged as the last in the abdominal series of soft cuticle receptors. In the isolated 6 A.G./rectum complex their output does not modify the movements of the hindgut. Thus the anal receptors may be non-specific, only responding to anal movements because of their position. In addition they are well placed to respond to telson flexion and may be the anatomical manifestation of the telson movement receptors described by Barth (1964) in *Procambarus clarkii*. It is probable that the anal receptors correspond to those described as discharging onto ipsilateral and contralateral interneurones during pulling out of the anal valve of

Procambarus (Wiersma and Hughes, 1961). The proximal terminations of these interneurones are as yet unknown. They must run at least as far as the third abdominal ganglion since Wiersma and Hughes recorded from the 3–4 connectives and it is possible that they may terminate in the brain. The soft cuticle receptors may well subserve a multiplicity of functions due to their apparent lack of specificity.

Although it has not been possible to demonstrate the presence of receptors innervating the hindgut physiologically, their presence has been demonstrated by histological methods on several occasions (Alexandrowicz, 1909; Janisch, 1923; Orlov, 1926). It is just possible that the fine dendritic processes of these cells innervate the proctodaeal cuticle in much the same way as the cells described above innervate the ventral soft cuticle of the abdomen. This supposition is reinforced by the fact that the proctodaeal cuticle is an invagination of the ventral soft cuticle which forms the anal lips. In addition, the longitudinal muscle strands insert into the proctodaeal cuticle as has been demonstrated by Cattaneo (1888) and Janisch (1923). Cuticular deformation must thus be a concomitant of hindgut movements. However, even if such receptors do occur their presence or absence may not affect the motor discharge along the P.I.N.'s, *at the level of the* 6 *A.G.* Such receptors could pool their input onto a common interneurone terminal, possibly in association with the input from the anal lips. The receptor discharge is not thought to be caused by the presence of faeces in the hindgut (see below) although it would probably occur in response to defaecatory movements. The interneurone(s) responding to this afferent information could easily transmit a patterned discharge forward to the brain. The rhythmic contractions of the intestinal muscles might well cause a constant receptor barrage to impinge on anterior going interneurones. Such an input into the brain would maintain the excitatory state of neurones causing hindgut movements. Any additional input, from whatever source, might then be sufficient to activate these central interneurones and thus the motor network supplying the hindgut.

In addition to the receptors described above, Orlov (1926) has indicated the presence of pyloric sensory cells whose dendrites innervate the hindgut after passing posteriorly from the pyloric region of the stomach. The axons of these cells enter the commissural ganglion. Three types of receptors are thus thought to innervate the hindgut: ventral soft cuticle receptors, proctodaeal cuticle receptors and pyloric sensory cells. None of these modulate the motor activity to the hindgut at the level of the 6 A.G. All may be centrally represented in the tritocerebral region of the brain.

Stimulation of the ipsilateral P.I.N.a. is followed by an output in the contralateral P.I.N.a. (see Figure 10). However, this is not thought to be due to a reflex arc between the hindgut and the 6 A.G., since even the slowest of the responses shown in Figure 10 indicates a pathway conducting at 2–3.5 metres/sec., whilst Pabst and Kennedy demonstrate that the maximum conduction velocity in the soft cuticle receptors which they describe is only 1.5 metres/sec. Other reductions in the overall conduction velocity would occur at any points of synaptic interaction and our conclusion is that the units recorded in the contralateral P.I.N.a.'s are due to the presence of bifurcating motor axons. Stimulation of one branch of such an axon could well produce an action potential capable of invading all its other terminals. We have reported the anatomical presence of such axons above.

From evidence presented by Herrick (1895) it seems that lobsters forage for food at night and are comparatively inactive during the day. This is the opposite of the situation in the gastropod mollusc, *Aplysia californica*, in which Strumwasser (1967) has demonstrated the presence of a parabolic burster neurone, in the parieto-visceral ganglion, which acts as an endogenous oscillator. In the absence of all synaptic input it emits spontaneous bursts followed by periods of silence. In isolated parieto-visceral ganglion preparations from animals exposed to several cycles of photoperiod (12 hours light followed by 12 hours darkness) the parabolic burster shows a large peak of impulse rate around the projected dawn. Similar types of neurones may occur in many other phyla, and are probably responsible for the persistent diurnal rhythmicity of *Astacus*, *Orconectes*, *Procambarus* and *Cambarus* (Brown, 1961), when these types are kept in constant darkness. The role of such endogenously active neurones might be to cause arousal in previously quiescent animals. If they exist in lobsters they could initiate foraging and feeding among other activities. Receptors of the stomach and mouthparts (Dando and Laverack, 1969; Laverack and Dando, 1968) may then impose modulation of the command neurones in the brain which control the hindgut.

In view of Morris and Maynard's (1970) investigations, in which the activity of the stomatogastric system was suspected to be controlled from higher centres, and our suggestion that the hindgut is controlled from the brain, it now seems possible that the presence of a centre co-ordinating all intestinal movements may be found, probably in the tritocerebrum. This suggests that the processes of feeding and defaecation are entirely automatic and interdependent, but we have observed that lobsters which have been starved for many weeks still defaecate. Perhaps the defaecatory response is driven by some cerebral oscillatory mechanism whose activity is temporally modulated by feeding. The absence of a receptor discharge during artificial dilation of the rectum suggests that faeces entering the rectum do not cause a reflexive output from the 6 A.G. to promote the defaecatory response. In fact it is thought, from observations on many lobsters, that faeces are always present in the rectum and midgut and are bound together in a mucous string. Defaecation would then simply drag this string further through the midgut, which is only weakly contractile (Yonge, 1924), and push its posterior end out

through the anus. Lobsters maintained in aquaria often have short strings of faeces hanging from the anus and these may remain there for several hours.

According to Horridge (1968) "the normal pattern of a sequence of motor impulses need not depend on feedback from the periphery" especially in invertebrates. Normal patterning (though often at reduced frequencies) in the absence of afferents has been demonstrated on many occasions (Pasztor, 1969; Burrows and Willows, 1969; Davies, 1969; Wilson, 1961; etc.). The defaecatory response of the lobster is also a centrally derived phenomenon.

Acknowledgements

We would like to thank Messrs. C. J. Roemmélé, J. Stevenson and R. Jack for technical assistance; also Dr. D. Graham for proof reading the paper.

References

Alexandrowicz, J. S. (1909). Zur Kenntnis des sympathischen nervensystems der Crustaceen. *Jena. Z. Naturw.* **45,** 395–444.

Allen, J. (1894). Studies on the nervous system of Crustacea. 1. The stomatogastric system of *Astacus* and *Homarus*. 2. On the beading of nerve fibres and on end-swellings. *Quart. Jl microsc. Sci.* **36,** 483–498.

Atwood, H. L., Hoyle, G. and Smyth, T. (1965). Mechanical and electrical properties of single innervated crab-muscle fibres. *J. Physiol.* **180,** 449–482.

Barth, F. G. (1964). A phasic-tonic proprioceptor in the telson of the crayfish *Procambarus clarkii* (Girard). *Z. vergl. Physiol.* **48,** 181–189.

Brown, F. A. (1961). Physiological rhythms. In *The Physiology of Crustacea*, **2,** Ed. T. H. Waterman, Academic Press, New York.

Burrows, M. and Willows, A. O. D. (1969). Neuronal co-ordination of rhythmic maxilliped beating in brachyuran and anomuran crustacea. *Comp. Biochem. Physiol.* **31,** 121–136.

Campbell, G. and Burnstock, G. (1968). Comparative physiology of gastro-intestinal motility. In *Handbook of Physiology. Alimentary canal*, **4,** Ed. C. T. Code, American Physiological Society, Washington.

Cattaneo, G. (1888). Sulla strutura dell intestino dei crostacei decapodi e sulle funzione delle loro glandule enzimatiche. *Atti. Soc. Italian Sc. Natur.* **30,** 238–272.

Dando, M. R. and Laverack, M. S. (1969). The anatomy and physiology of the posterior stomach nerve (p.s.n.) in some decapod crustacea. *Proc. R. Soc.* **171,** 465–482.

Davis, W. J. (1969). Reflex organization in the swimmeret system of the lobster. 1. Intra-segmental reflexes. *J. exp. Biol.* **51,** 547–563.

Ebara, A. (1969). Spontaneous activity of crayfish intestine. *Annotnes. zool. jap.* **42,** 169–175.

Herrick, F. H. (1895). The American lobster: a study of its habits and development. *Bull. U.S. Fish Commn.* **25,** 1–252.

Horridge, G. A. (1968). *Interneurons.* Freeman, London.

Hoyle, G. (1967). Specificity of muscle. In *Invertebrate Nervous Systems*, Ed. C. A. G. Wiersma, University of Chicago Press, Chicago.

Janisch, E. (1923). Der Bau des Enddarms von *Astacus fluviatilis* (*Potamobius astacus*). Ein Beitrag zur Morphologie der Dekapoden. *Z. wiss. Zool.* **121,** 1–63.

Johansson, A. S. and Schreiner, B. (1965). Neurosecretory cells in the ventral ganglia of the lobster, *Homarus vulgaris* L. *Gen. comp. Endocr.* **5,** 558–567.

Keim, W. (1915). Der Nervensystem von *Astacus fluviatilis* (*Potamobius astacus* L.). *Z. wiss. Zool.* **113,** 485–545.

Krieger, K. R (1880). Ueber das central Nervensystem des Flusskrebses. *Z. wiss. Zool.* **33,** 527–594.

Krohn, A. D. (1834). Ueber die Verdauungsnerven des Krebses. *Isis oder Encyclopädische Zeitung von Oken* **5,** 529–531.

Larimer, J. L. and Kennedy, D. (1969). Innervation patterns of fast and slow muscle in the uropods of crayfish. *J. exp. Biol.* **51,** 119–133.

Laverack, M. S. and Dando, M. R. (1968). The anatomy and physiology of mouthpart receptors in the lobster, *Homarus vulgaris*. *Z. vergl. Physiol.* **61,** 175–195.

Lemoine, V. (1868). A l'histoire des systèmes nerveux musculaire et glandulaire de l'écrevisse. *Annls Sci. nat. (Zool)* **9,** 99–280.

Maynard, D. M. (1966). Integration in crustacean ganglia. *Symp. Soc. exp. Biol.* **20,** 111–149.

Miller, F. R. (1910). On the rhythmical contractility of the anal musculature of the crayfish and the lobster. *J. Physiol.* **40,** 431–444.

Morris, J. and Maynard, D. M. (1970). Recordings from the stomatogastric nervous system in intact lobsters. *Comp. Biochem. Physiol.* **33,** 969–974.

Orlov, J. (1926). Système nerveux intestinal de l'écrevisse. *Izv. biol. nauchno-issled. Inst. biol. Sta. perm. gosud. Univ.* **5,** 29–32.

Pabst, H. and Kennedy, D. (1967). Cutaneous mechanoreceptors influencing motor output in the crayfish abdomen. *Z. vergl. Physiol.* **57,** 190–208.

Pantin, C. F. A. (1964). *Notes on Microscopical Technique for Zoologists.* Cambridge University Press, Cambridge.

Pasztor, V. M. (1969). The neurophysiology of respiration in decapod crustacea. 2. The sensory system. *Can. J. Zool.* **47,** 435–441.

Police, G. (1908). Sul sistema nervoso viscerale dei crostacei decapodi. *Mitt. zool. Sta. Neapel* **19,** 69–116.

Prosser, C. L., Nystrom, R. A. and Nagai, T. (1965). Electrical and mechanical activity in intestinal muscles of several invertebrate animals. *Comp. Biochem. Physiol.* **14,** 53–70.

Retzius, G. (1890). Zur Kenntnis des Nervensystems der Crustaceen. *Biol. Unters.* **1.**

Schmidt, W. (1915). Die Muskulatur von *Astacus fluviatillis* (*Potamobius astacus* L.). *Z. wiss. Zool.* **113,** 165–251.

Shelton, R. G. J. and Laverack, M. S. (1968). Observations on a redescribed crustacean cuticular sense organ. *Comp. Biochem. Physiol.* **25,** 1049–1059.

Strumwasser, F. (1967). Types of information stored in single neurons. In *Invertebrate Nervous Systems*, Ed. C. A. G. Wiersma, University of Chicago Press, Chicago.

Wiersma, C. A. G. and Hughes, G. M. (1961). On the functional anatomy of neuronal units in the abdominal cord of the crayfish, *Procambarus clarkii* (Girard). *J. comp. Neurol.* **116,** 209–228.

Wilson, D. M. (1961). The central nervous control of flight in a locust. *J. exp. Biol.* **38,** 471–490.

Winlow, W. (1970). The control of hindgut movements in the lobster, *Homarus gammarus* (L.). Ph.D. Thesis, University of St. Andrews.

Winlow, W. and Laverack, M. S. (1970). The occurrence of an anal proprioceptor in the decapod crustacea *Homarus gammarus* (L.) (Syn. *H. vulgaris* M. Ed.) and *Nephrops norvegicus* (Leach). *Life Sciences* **9,** 93–97.

Winlow, W. and Laverack, M. S. (1972). The control of hindgut motility in the lobster *Homarus gammarus* (L.). 2. Motor output. *Mar. Behav. Physiol.* **1,** 29–47.

Yonge, C. M. (1924). Studies on the comparative physiology of digestion. 2. The mechanism of feeding, digestion and assimilation in *Nephrops norvegicus*. *Br. J. exp. Biol.* **1,** 343–389.