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OBSERVATIONS ON THE ELECTRICAL AND MECHANICAL PROPERTIES OF THE MYOTOMES OF THE LANCELET (BRANCHIOSTOMA LANCEOLATUM)

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INTRODUCTION

Over the last decade considerable advances have been made in our understanding of the fine structure, electrical properties and neural activation of the trunk musculature of fish (Barets, 1961; Jansen, Andersen, and Loyning, 1962; Bone, 1966; Hudson, 1969; Roberts, 1969). Apart from the work of Geduldig (1965) on the permeability to potassium and the resting potential, there is no information on the physiology of the myotomes of Amphioxus. It seems reasonable to hope that studies on lancelet myotomes would throw some light on the origin of vertebrate trunk muscles, and in particular on the dual system of slow and fast muscle fibres in fish.

In 1966 Flood showed, by means of electronmicrographs, that in common with nematodes and echinoderms Amphioxus possesses muscle fibres which make contact with the nerve cord by means of fine cytoplasmic extensions or muscle tails. The 'ventral roots' described by earlier authors are composed of these muscle tails. In this and a later communication in 1967 Flood was also able to show that the myotomes contained three morphologically distinct types of muscle fibre or lamella. The deep and the superficial lamellae corresponded well with the fast and slow fibres of fish in terms of position, fine structure and histochemistry. Furthermore Guthrie (1967) showed that slow and fast contractions could be evoked separately from the myotomes by local electrical stimulation of the spinal cord. The work described here seeks to extend these findings.

METHODS

Pieces of lancelet trunk musculature were mounted in a Perspex perfusion bath generally similar in design to the one figured by Usherwood & Machili (1968) for insect nerve-muscle preparations. Sea water in which exposed tissues of Amphioxus survive well was used as the perfusion medium. The water was oxygenated and cooled in an ice chamber to below 18 °C. When microelectrode and tension records were required a small three-pronged rake was used to immobilize the chosen myotome, which was also impaled by the hook from a force displacement transducer. This arrangement is shown in Fig. 1*c*.

Glass microelectrodes were pulled having tip diameters between 0·2 μ and 0·4 μ.

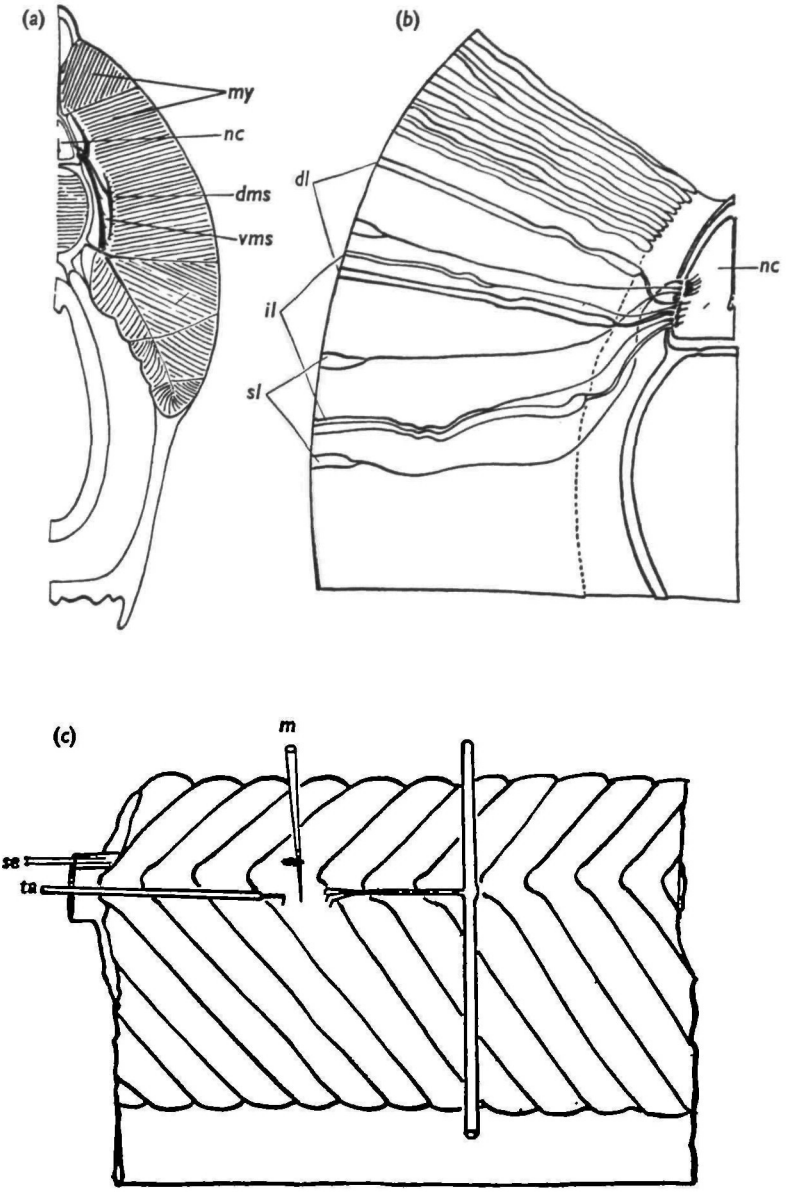

Fig. 1. (*a*) Diagram of the sagittal half of a transverse section through a lancelet to show: parts of adjacent myotomes, *my*; the nerve cord, *nc*; the dorsal muscle strand, *dms*; the ventral muscle-tail strand, *vms*. (*b*) Diagrammatic representation of part of a myotomal field to show the three kinds of muscle lamellae: *sl*, superficial lamellae; *dl*, deep lamellae; *il*, intermediate lamellae; *nc*, nerve cord. (*c*) lateral view of a piece of trunk musculature arranged for mechanical and electrical recording; *m*, microelectrode; *se*, stimulating electrode in the nerve cord; *ta*, transducer arm and hook. The stippled area shows the position of the neuromuscular synapses.

(established by electron microscopical examination of a similar batch of electrodes). When filled with electrolyte their resistance did not exceed 40 MΩ.

Initial experiments showed that KCl-filled electrodes were associated with a rapid decline in the value of the resting potential and therefore sodium chloride, sodium acetate and the dye Fast Green (FCF) were tested as electrolytes. NaCl was eventually used in almost all experiments.

RESULTS

The structure of the myotomes

A transverse section through the pharyngeal region of Amphioxus reveals the lamellar nature of the myotomes, their orientation, and the position of the muscle-tail strands (Fig. 1*a*). Under the light miscrocope it is just possible to see individual lamellae.

Flood (1966, 1967) showed that the muscle lamellae were of three main types. (i) Superficial lamellae, approximately 2 μ thick and extending about 10 μ inwards from the surface of the myotomes. These are sarcoplasm-rich fibres with large amounts of glycogen. (ii) Deep lamellae, approximately 1 μ thick which may extend the full depth of the myotome, that is to say 300 μ or more. These form the bulk of the myotome and contain little sarcoplasm or glycogen. Near the midline some of the deep lamellae may expand to a thickness of 4–5 μ. (iii) Intermediate lamellae intermediate between deep and superficial lamellae in terms of amount glycogen and sarcoplasmic material. They may extend from the surface to near the centre of the myotome, and are very thin (0·5 μ). These different types of lamellae are shown diagrammatically in Fig. 1*b*.

A striking feature is that Flood's electronmicrographs show many tight junctions or zonulae occludentes between adjacent lamellae of the same or of a different type and also between the muscle tails. Similar junctions were found by deBell, del Castillo and Morales (1967) between muscle-tail terminals in *Ascaris*.

Mechanical responses

If the myotome is stretched by small increments of length up to 150% of the relaxed length of the lamellae, and mechanical responses to a standard stimulus are evoked after each increment, the height of the contraction increases progressively. It is not feasible to stretch the myotome by more than 50% of its length without tearing its insertions on the myocommata, and measurements of increases in the length of the myotome when the animal is severely flexed (130°) indicate that the myotomal length does not increase by more than 25–30% of the relaxed length under these conditions. This is presumably associated with the large number (60) of very narrow myotomes into which the trunk musculature of the lancelet is divided. The term 'relaxed length' is used here to describe the length of the quiescent myotome measured at the inflexion (see Fig. 1*c*), when the body axis is quite straight. For recordings the myotome was normally stretched by 25–30%. Unless otherwise stated stimulus pulses were always applied to the nerve cord as indicated in Fig. 1*c*.

The maximal response to a single shock is a 'fast' twitch which sends to obscure any slow response, as the amplitude of the slow response is usually less than 10% of

the fast response in the previously fresh, unstimulated myotome. A myotome from the middle of the body is about 1 mm long at its inflexion and produces a maximum tension of 150–200 mg. The fast twitch has a rise time of 70–80 msec., and falls to near zero tension in about 120 msec., as can be seen from Fig. 2*a*.

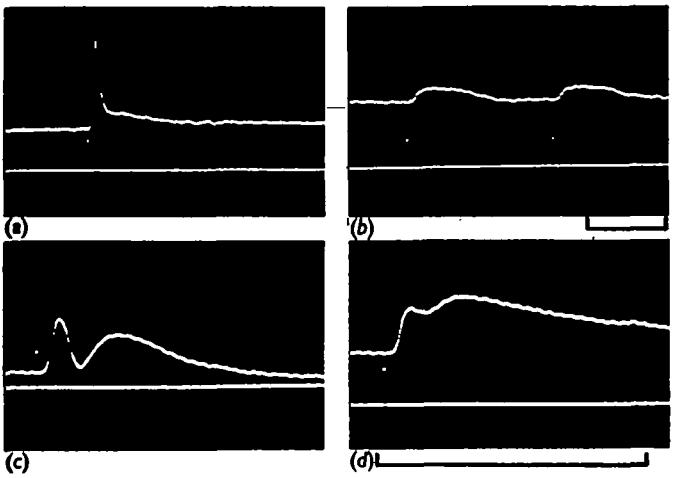

Fig. 2. Mechanical responses of the myotome to stimulation of the nerve cord. (*a*) Compound response; largely fast. (*b*) Slow response revealed by repetitive stimulation (*c*) Compound response with delayed slow phase; deep insertion of transducer hook. (*d*) Compound response; middle layers of the myotome. High-intensity stimulation. Time scale 500 ms.

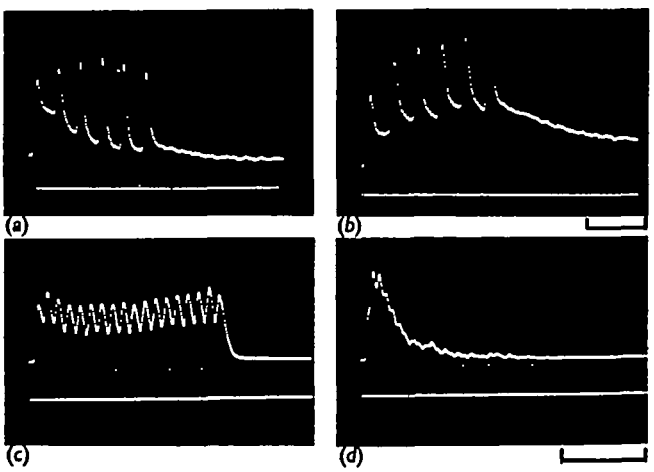

Fig. 3. Mechanical responses. (*a*) and (*b*) Responses to a stimulus frequency of 5Hz. In (*a*) the slow component appears initially, but then declines. In (*b*) the slow contraction summates at successive responses. (*c*) Onset of summation of the fast response at 15 Hz.(*d*) Typical high-frequency response to a stimulus frequency of 30 Hz. Rapid decline in response amplitude after the first three pulses. Time scales 500 ms.

With repetitive stimulation the fast response fatigues and the relaxation phase can be seen to include a separate slow contraction. Both contractions are responses to a single shock and may therefore be described as a slow and a fast twitch respectively.

A slow and a fast twitch response, with in each case a similar time course and a similar relation to fatigue, have been described by Jansen *et al.* (1962) in the muscles of *Myxine*. By altering the position of the stimulating electrodes, the pulse length and the stimulus intensity it is possible to evoke responses which are largely fast or slow. It is also possible to delay the slow response by lodging the transducer hook in the deeper layers of the myotome. A variety of slow and fast responses are illustrated in Fig. 2. The slow twitch rises to a maximum in about 250 msec. and declines to near zero tension in 500–700 msec.

A few of the responses of the myotome to repetitive stimulation are shown in Fig. 3. The degree to which the slow system is activated determines to a large extent the proportion of the total tension appearing as a fused or non-repetitive response, but at 25–30 Hz the reduction of the repetitive response to less than 10% can be accounted for by summation of the fast contractions alone. Between 6 and 7 Hz the muscle no longer responds to the whole of a stimulus train if this is prolonged for more than 2 sec., while at a stimulus frequency of 40 Hz the mechanical response is only maintained for 100–150 msec. when the period of stimulation is 2 sec. This high-frequency fatigue is illustrated in Fig. 3*d*. The increase in tension with increased frequency of stimulation is not nearly so striking as it is in the notochordal muscles (Guthrie & Banks, 1970), amounting to 1·5–2·0 times the maximal isolated fast twitch at 40 Hz.

Attempts were made to obtain separate fast and slow repetitive responses to a range of stimulus frequencies, by starting with either fresh or fatigued muscles, but the rapid recovery of the fast response makes this difficult. Where the slow component is small in responses to isolated shocks, repetitive stimulation produces only a gradual increase in the size of the fused response, and this fusion plateau remains level at low frequencies as in the response shown in Fig. 3*c*. Sometimes a slow component appears and then suddenly drops away, demonstrating, as in Fig. 3*a*, that a stimulus frequency of 5 Hz does not produced any summation of the fast response. Where on the other hand the slow component is relatively large at the outset, and has been accentuated by low-frequency repetitive stimulation (1 Hz), raising the frequency to 5 Hz produces a marked summation of the slow response. This is illustrated in Fig. 3*b*. As could be predicted from their relaxation times, summation begins to be noticeable at frequencies above 1 Hz for the slow twitch and at about 7 Hz for the fast twitch response. The slow response when recorded with the transducer hook inserted to the fullest extent in the myotome often shows a marked delay as compared with the fast component. This can be seen in Fig. 2*c*. Compared with the normal compound response recorded near the surface, the fast component is 5–10 msec. earlier, and the slow component may be delayed by as much as 20 msec.

Under these conditions there is a closer contact between the transducer hook and the deep lamellae that in the case of the superficial lamellae, and this may be the reason for the delay in the registration of mechanical events in the outer region of the myotome; it would refer the slow contractions to the superficial lamellae. In the fatigue myotome the height of the slow response compared to the fast phase varied from 60% to 180% with the depth of the transducer arm in the myotome, the higher values deriving from the middle of the myotome, the lowest from the outer layers. This might occur if the slow contractions were produced by elements lying at mid-depth in the myotome, but microelectrode recordings (described later) suggest instead that these

unusually large slow contractions involve a fast component, and therefore they do not enter into the question of the origin of the slow response.

The problem of synchrony of contractions seemed interesting in relation to the absence of a fast pathway in the form of motor nerves and the unusually long ventral arm of the myotome. We were prepared to find nevertheless that the abundance of tight junctions between the lamellae ensured very small differences in latency. In fact contractions commenced in the muscle fibres near the ventral root areas 12–16 m-sec. after the rising phase of a shock applied to the nerve cord opposite the ventral root, 16–20 msec. later in the dorsal arm of the myotome 0·9 mm. away, and 28–40 msec. later in the extremity of the ventral arm 3.6–4·0 mm. away. The differences in these conduction times must be those involved in transmission through the muscle-tail strands, and suggests a conduction rate of 0·25 m./sec (12 measurements). This may account for the discrepancy between the rise time of a fast twitch involving most of the fibres in the myotome (70–80 msec.) and a very localized twitch produced by stimulation in the myotome with a micropipette (30 msec.). This method incidentally demonstrated similar latencies to those derived from nerve-cord stimulation. The delay in the onset of contraction in the extremities of the myotome may explain the acute inflexion of the myotome which results in the anterior border of the ventral extremity of one myotome lying on the same vertical line as the most anterior boundary of the fourth myotome posterior to it. Thus flexion of the body in a particular vertical plane during swimming will require contractions in different parts of four adjacent myotomes. It can be seen that nerve-cord conduction (Guthrie, 1967) delays at the neuromuscular synapse (see later sections) and conduction in the muscle tails introduce about the right order of delays to ensure a synchronous contraction at the same level.

Local thresholds to direct stimulation within the myotome were investigated. Does the occurrence of many tight junctions allow the myotome to be activated from the periphery? Stimulating the superficial lamellae electrically by means of a 10 μ micropipette suggests that this is not so. Stimulus intensities just sufficient to produce contractions do not spread far from the electrode, but the same stimulus applied near the ventral root area results in large responses which are also widespread. Very small adjustments of electrode position within this area make a considerable difference to the size of contractions.

Resting potentials and spontaneous activity

Altogether 563 measurements of resting potential were made from the myotomal lamellae, and these provided a mean value of 48·94 mV. The standard deviation of the sample was $\pm$ 15·5 mV.

This value differs considerably from the mean of Geduldig's (1965) resting potentials from the lancelet myotome, which was 57·5 mV. when the muscle was bathed in an artificial sea water containing 9 mM/l. of potassium.

There are a number of possible reasons for this discrepancy. We found a considerably higher mean value when the preparations were examined in uncirculated sea water than when the preparations were pinned out in a bath that was part of a flow system. Sixty-six penetrations made under the former conditions yielded a mean of 59·2 mV.

There were also considerable differences in the resting potentials observed at different depths in the myotome. The first 322 penetrations were all made by traversing the microelectrode from points near the inflexion of the myotome and just below the skin to the region of the notochord or the nerve chord, that is to say, in the horizontal transverse plane of the animal. The results were divided between those observed near the surface, those from mid-depth and those from areas near the notochord. Accurate

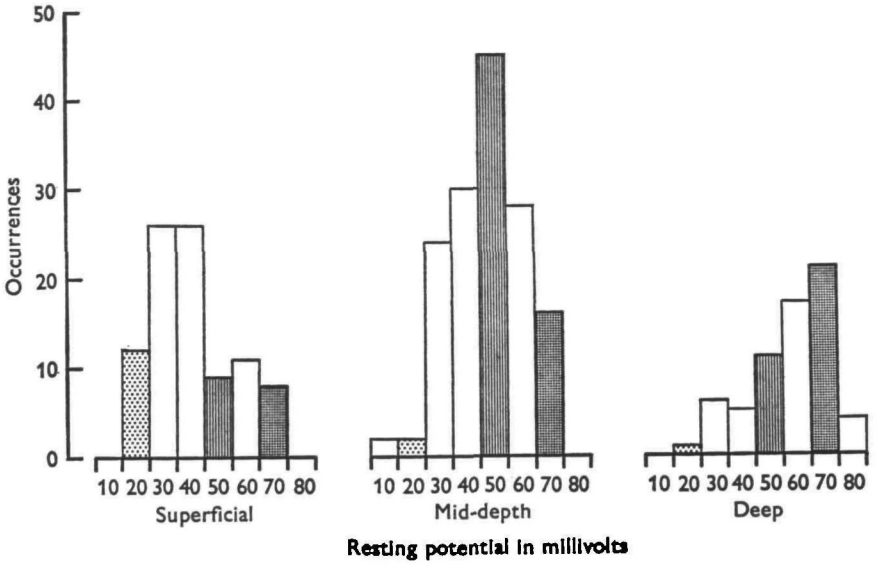

Fig. 4. Histograms showing the distribution of 322 resting potentials recorded at three different levels within the myotome.

gauging of the depth proved difficult because of the impossibility of knowing when the electrode touched the surface of the myotome, as this is not always accompanied by the registration of a resting potential. The mean values for the three groups and the numbers in each sample were as follows: (i) surface, 39·46 mV. (93); (ii) mid-depth, 46·36 (161); (iii), deep, 56·85 mV. (68). A *t* test was applied to these samples to see whether there was a significant difference between superficial and mid-depth values, and between mid-depth and deep values, and in both instances the probability of these values deriving from the same population was less than 0·001.

The possibility that the increase in resting potential with depth was due to blockage of the electrode tip was examined by checking the tip potential periodically. In only a few instances was there any increase in tip potential even after many lamellae had been penetrated.

To check on the existence of this potential profile the microelectrode was traversed in the horizontal plane, but from the mid line to the skin, and also in the vertical plane starting from the dorsal surface. The results of these penetrations largely confirmed the earlier findings. It should be noted that Geduldig's method involved dissecting off a piece of myotome and penetrating it with the microelectrode from the inner side so that perhaps his mean value of 57·5 mV. should be compared to ours for the deep lamellae, i.e. 56·85 mV. His statement that only 15 measurements were made from each piece suggests that only the lamellae near the exposed surface were sampled.

These observations indicate that there is a potential profile across the myotome, and therefore these three groups mentioned earlier are presented in the form of a histogram in Fig. 4.

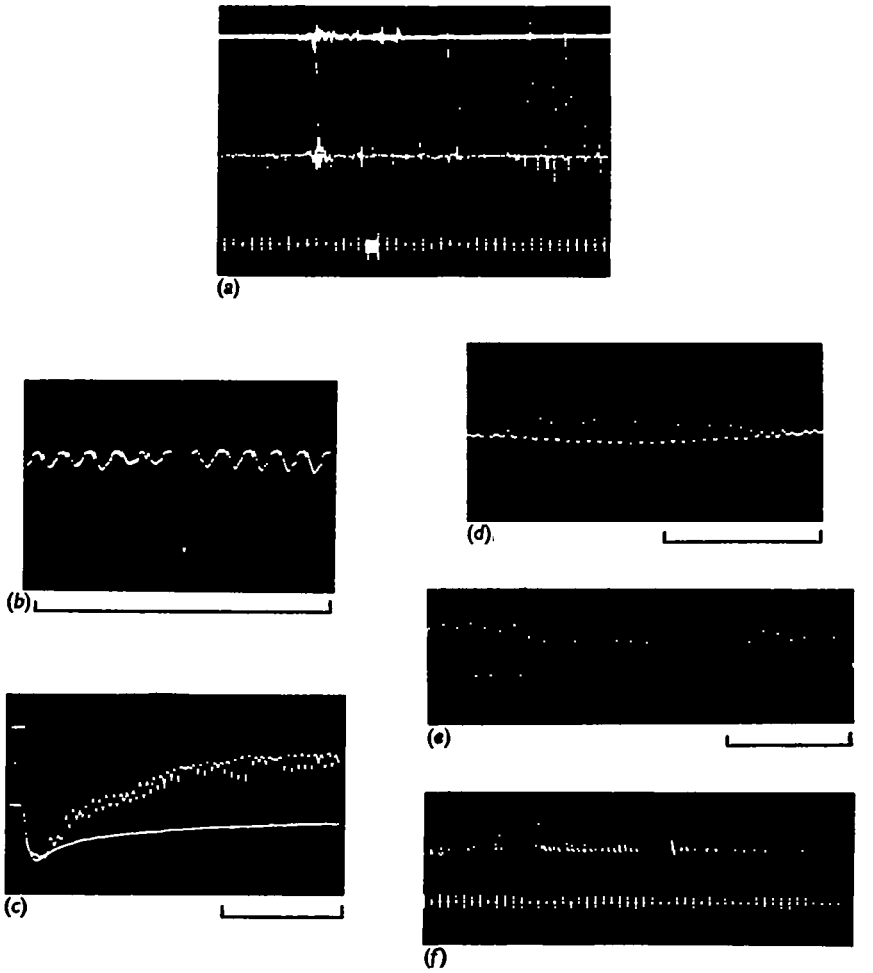

Fig. 5. Recordings of spontaneous potentials (*a*) extracellular, the rest intracellular. (*a*) Recordings from the nerve cord (upper trace), and an adjacent myotome (lower trace).Bursts of potentials in the nerve cord are reflected in the myotome, but many of the large myotomal potentials are independent of activity in the nerve cord. Mechanical stimulation at filled rectangle on the time trace. (*b*) Negative spontaneous potentials. (*c*) A drift from −50 mv. to about 10 mV. in the resting potential is accompanied by a reversal of polarity of the spontaneous potentials at about −25 mv. The other trace shows tension, maximum downwards. (*d*) Positive spontaneous potentials. (*e*) Spontaneous potentials at 30 % maximum resting potential. (*f*) Spontaneous potentials displayed as in (*a*). Horizontal line, zero potential. Resting level, 52 mV. Time scales: (*a*) and (*f*) 1sec., (*b*) 100 msec., (*c*) (*d*) and (*e*) 500 msec.

The question of the identification of the three types of lamellae with particular resting potential values must remain an open one because of the great range and variability of these resting potentials. The potential gradient does not correspond in any direct way with the distribution of the three types of lamella described by Flood.

Damage to the skin during dissection and osmotic effects accentuated by circulating sea water may be associated with low resting potentials at the surface of the myotome, but cannot explain differences between the mid-depth and deep layers. It is clear, however, that the deep lamellae which constitute over 90% of the muscle by volume are likely to have a distribution of values similar to those of our sample.

Recordings made with fine wire electrodes from the nerve cord and the myotomes showed that while muscle twitches were accompanied by bursts of potentials at both recording sites, large potentials occurred in the myotomes without any sign of neural activity. These spontaneous potentials occurred fairly regularly at rates of one every 1–4 sec. The animals were lying in a completely relaxed or quiescent state. Dead lancelets are noticeably more flaccid than quiescent ones, and this may be associated with the loss of a maintained tonus. Some myotomes show a very high level of spontaneous activity, others very little. A recording made with external leads is shown in Fig. 5*a*.

Microelectrode penetrations of the lamellae immediately demonstrated trains of spontaneous potentials at all levels within the myotome. These potentials varied in amplitude from 5 to 30 mV., and occurred in rhythmic bursts of 10 to 20 potentials repeated once every 1–5 sec. Within a burst the frequency of potentials was between 10 and 20 per sec. The shape and polarity of these potentials varied considerably, and some of them are shown in Fig. 5*c*. The rise time of a spontaneous potential was between 10 and 20 msec. If intracellular and extracellular records are compared it can be seen that the large extracellular potentials correspond to the bursts obtained with the microelectrode, and if as shown in Fig. 5*f* the intracellular recording is displayed in the same way as the external one the bursts appear as single large potentials.

The polarity of the spontaneous potentials varies with the resting potential level, reversing from positive to negative at about 60% of the maximum resting potential for that fibre. Where there is a rapid drift in the resting potential this reversal can be seen clearly, as in Fig. 5*c*. The magnitude of the spontaneous potentials does not diminish appreciably as the potential level drifts from 50 mV. to 10 or 15 mV., but declines abruptly beyond this point. Near the reversal point the spontaneous potentials may take on a sinusoidal appearance, at least for short periods of time, as shown in Fig. 5*e*. Farley (1964) has produced oscillograms of remarkable similarity to these spontaneous potentials by a computer simulation technique in which various irregularities are faithfully reproduced. The type of model that was used by Farley was a loosely coupled isotropic network, and its significance will be examined in the discussion.

Very small mechanical changes could sometimes be observed during particularly intense electrical activity in the myotome, but they were too small for systematic study.

The spontaneous potentials in the muscles of *Ascaris* described by de Bell, del Castillo and Sanchez (1963) are generally similar to those observed in the lancelet. However, they occur at a slightly lower frequency and fall more clearly into small potentials (3–5 mV.), and spikes (15–30 mV.) which may have a positive overshoot as the mean resting potential was only 29·4 mV. These authors were able to show that the spontaneous potentials originated in the region of muscle-tail terminals where the tight junctions occurred.

Electrical responses

During a reflexly evoked twitch, extracellular recordings made with fine silver wire leads reveal a burst of small potentials in the myotome. Their highest frequency corresponds roughly with peak tension in the muscle (Guthrie, 1967). Later work has suggested that this may be an atrial stretch-receptor discharge, as the axons from these sense cells run in the adjacent myocommata, but it was not possible to pursue this further at the time. A record of this kind of burst is shown in Fig. 5*a*.

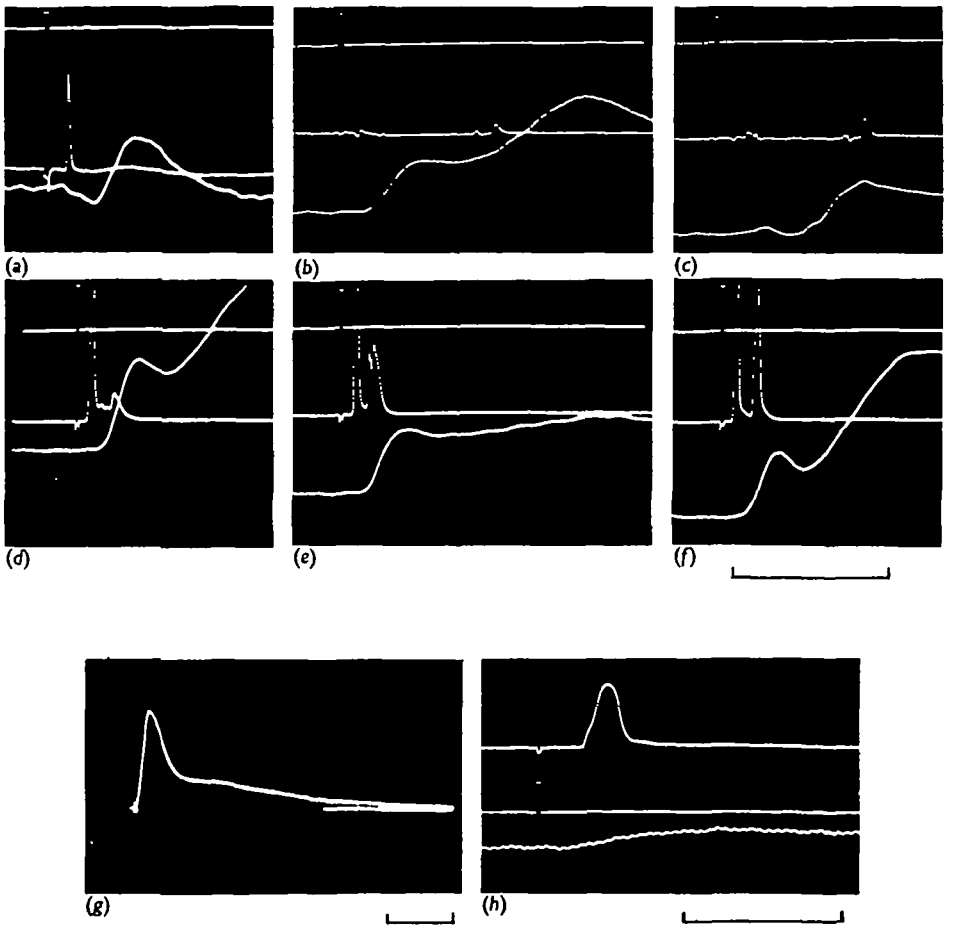

Fig. 6. Electrical and mechanical responses. The upper trace in (*d*) to (*f*) marks zero potential and shows the stimulus pulse, the second trace shows the electrical response, the third trace the mechanical response. (*a*) The fast response. (*b*) and (*c*) small potentials accompanying the slow component of a compound mechanical response. (*d*) to (*f*) The development of the second fast spike. (*g*) Extracellular recording made during a compound mechanical response. (*h*) Anomalous slow spike. Recording as (*a*) to (*f*), but second trace, stimulus trace. All time scales 100 msec., except (*g*) 10 msec. Some spikes retouched.

If the recording electrode is a glass capillary with a tip diameter of 10–20 μ filled with 3 M-KCl, a different kind of electrical response can be observed, and this is illustrated in Fig. 6*g*. At first sight it appears to be a reflexion of the mechanical changes

during a normal contraction, but the initial rise precedes that of the mechanical response by 10 msec., and the peak of the slow phase is 20–50 msec. in advance of the similar part of the slow twitch. An electrode with a diameter of 15 μ would be expected to record potential changes in 15 or 20 lamellae, and the combined signal might be smoothed by secondary fluctuations through the tight junctions. This type of record suggests that during the normal biphasic contraction closely grouped large potentials appear first in the lamellae followed by temporally dispersed small potentials.

The method employed for making intracellular recordings is indicated in Fig. 1, and has been mentioned in the section on resting potentials. The NaCl-filled microelectrode was traversed slowly through the myotome, and a shock was applied to the nerve cord when a steady resting potential was obtained. Less than 20 % of satisfactory impalements produced a transient depolarization even when accompanied by a mechanical response, and it seems probable that only a proportion of the lamellae are electrically active during most contractions.

The electrical events that accompanied mechanical responses fell into four main categories. (*a*) Large potentials with a total amplitude of 30–70 mV., and a duration of 10–15 msec. The largest may have a positive overshoot of 15–20 mv. They follow the stimulus artifact by about 10 msec., and precede the commencement of the fast twitch by 10–15 msec. Examples are shown in Fig. 6*a*, *d–f*. (*b*) Small simple potentials with a value of 1–10 mV., and a duration of 10–15 msec. These potentials mainly appear 70–90 msec. after the stimulus artifact, and between one and five may occur in response to a single shock. Their relation to the slow twitch is very variable. They may precede the commencement of the slow response by 10–40 msec., or may occur at any time during the rising phase of the contraction. These are illustrated in Fig. 6*b*, *c*. (*c*) Compound potentials. These, as can be seen in Fig. 6*e* are initial stages in the formation of a second spike-like response, but may be confused with class (*b*) potentials when they are small. (*d*) A rare category of potentials is occasionally observed at low resting potentials, having the appearance of very slow spikes. An example is given in Fig. 6*h*. The duration is that of a spontaneous potential—about 30 msec.

The most striking feature of the large class (*a*) potentials is that two of them may occur in response to a single shock separated by an interval of about 15 msec. If the compound mechanical response which accompanies the double spike-like response is examined it can be seen to belong to the type in which the slow phase is unusually large, and is characterized by a shorter rise time than normal. This can be seen in Fig. 2*d*. The slow phase in this case is associated with both class (*a*) and (*b*) electrical events. The second spike-like response does differ from the first in that it is a graded event, rather than an all-or-nothing potential. The absence of a fast twitch in response to the second spike does suggest that the fatigue so characteristic of the fast system is a property of the contractile mechanism rather than of the lamellar membrane or neuromuscular synapse.

Do these records present any information bearing on the function of the tight junctions? Those records, like the one in Fig. 6*b* which show the small potentials, also show very small deflexions with the latency characteristic of the fast or large potentials, as well as other small deflexions in the 0·5–2 mV. range which may derive from adjacent lamellae. It is possible of course that even the class (*b*) events are taking place in other lamellae and are merely reflected in an attenuated form in the lamellae from which

these records are taken. It might be suggested that the graded nature of the second spike owes something to local reinforcement of the type occurring in Farley's (1964) neuromime.

Another point concerns latency and synchrony in the neuromuscular pathways. The earliest electrical responses in the lamellae occur 10 msec. after the stimulus artifact even when the tip of the recording electrode is very close to the nerve cord. In the section on mechanical responses it was suggested that conduction in the muscle-tail strand at the rate of 0·25 m./sec., so that a delay of 10 msec., if it was due solely to conduction through the muscle tails would require this pathway to be 2·5 mm. long. This indicates that nerve-cord stimulation is occurring via presynaptic elements, and as the electrodes were close to the neuromuscular synapse this delay can be largely referred to delay at this synapse. Incidentally, deBell *et al.* (1963) showed that conduction in the muscle tails of *Ascaris* was three times slower (0·06 m./sec.) than in the lancelet. The delay in the appearance of the class (*b*) small potentials is perhaps associated with Flood's (1966) finding that the muscle tails of the superficial lamellae are approximately half the diameter (0·5 μ instead of 1·0 μ), and several times as long, as those of the deep lamellae. On the other hand the form of the dorsal and ventral neuromotor synapses may also be involved. Flood showed that the presynaptic boutons opposite the thick muscle tails consisted of a single layer of large profiles containing large vesicles, while the synaptic envelopes opposite the fine fibres were arranged in layers, were smaller, and contained smaller vesicles. The single-layer system of synapses is clearly one that is likely to be associated with a rapid synchronous response, rather than with a delayed progressive type of response.

From the evidence presented here it appears likely that the fast twitch response is associated with large potentials, the slow twitch with delayed small potentials.

Motor localization in the spinal cord

Pieces of lancelet were set up in a vertical position so that micro-electrodes filled with the dye Fast Green (FCF) could be traversed into the spinal cord along its main axis, as shown in the diagram in Fig. 7*a*, following the entry of an electrode into an area where stimulation produced contractions, a steady outward current at 5–10 μA. was passed until dye appeared at the stimulus site. The spot dye could be limited to an area 20–30 μ across, and often stained small groups of cells. Owing to the vertical orientation of the preparation it was difficult to set up the myotomes for the recording of mechanical responses, but this was done in a number of experiments.

For the purposes of this description the nerve cord was divided into five parts corresponding to the areas shown in the diagrammatic transverse section in Fig. 7*b*.

In the pharyngeal region (the atriopore to somite 6) brief shocks applied to areas 1 and 2 caused marked contractions in the myotome on the same side. Stimulation in areas 1, 2 or 3 never produced contalateral contractions. Most of these responses could be described as local fast twitches at intensities just above threshold. At higher intensities the contraction spread to several adjacent myotomes. Stimulation within area 3 produced rather weak local myotomal twitches to isolated shocks, but short trains of stimuli at 5Hz sometimes provoked powerful complex contractions. D.c. pulses with a duration of 5 sec. often produced strong complex contractions at the 'off'. These complex responses involved the pterygial musculature which is believed to be

connected with the median Rhode cell (Bone, 1960), and this lies in area 3. Stimulation of areas 4 and 5 was not effective in this region.

The post-atrial region resembled the pharyngeal region as far as most areas were concerned, with the following exceptions. Stimulation within areas 1 and 2 at frequencies

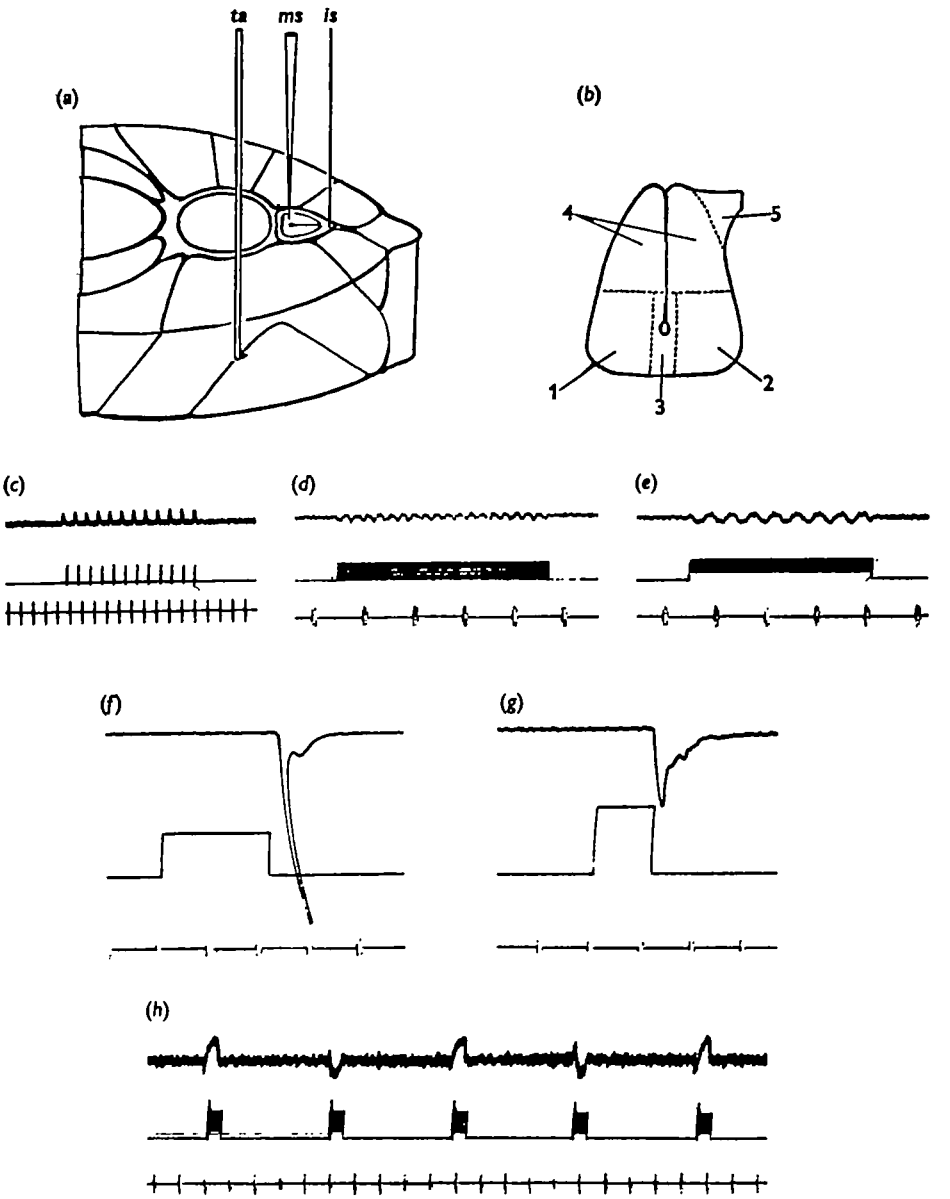

Fig. 7. Mechanical responses elicited by local stimulation within the nerve cord. (*a*) Arrangement of: the transducer arm, *ta*; microelectrode for stimulation, *ms*; indifferent electrode, *is*. (*b*) Areas of the nerve cord explored. (*c–h*). Responses to stimulation in the following areas: (*c*) Areas 1 and 2 in the pharyngeal region. (*d*) and (*e*) Areas 1 and 2 in the post-atrial region. (*f*) and (*g*) Areas 1 and 2 in the brain region (somites 1 to 6). (*h*) Area 5 in the post-atrial region. Stimulus frequencies (*d*) 50 Hz, (*e*) 100 Hz, (*h*) Bursts of 100 Hz. All time markers in seconds.

between 5 and 100 Hz produced rhythmic contractions involving the myotomes of both sides. The frequency of the contractions was always much lower than the stimulus frequency, and raising the stimulus frequency often lowered rather than raised the response frequency. Examples of these rhythms are shown in Fig. 7*d*, *e*. In one or two instances we were able to penetrate a dorsal root (area 5), and a cluster of dorsal root cells was clearly stained. In fig. 7*h* remarkable alternating response to repetitive stimulation of a dorsal root is shown, involving contraction in the ipsilateral myotome, and then in the contralateral myotome.It seemed possible that this was because of refractoriness in the centres controlling the myotomes of either side, but longer intervals between stimulus trains had only an initial effect in producing unilateral contractions.

It was hoped that stimulation of the brain area might yield interesting co-ordinated responses. This area extending back to the cell body of the median Rhode cell is of great complexity with many cell types, and comprises several distinct regions. The giant-cell area between dorsal roots 1 and 3 proved inexcitable, but stimulation between dorsal roots 4 and 6 elicited compound responses in the myotomal and the pterygial muscles. The stimulus was a 5 sec. d.c. pulse applied to areas 1 and 2 and the response was ipsilateral. Because the response occurred at the 'off' when the pulse was positive, attempts were made to reproduce this at the 'on' of a negative pulse but this was unsuccessful.

These results point to one or two tentative conclusions. Co-ordination of myotomal contractions of opposite sides may depend on centres in the dorsal rather than in the ventral regions of the cord. The tail region of the cord contains autonomous centres little influenced by the brain area, and the anterior giant-cell area of the brain may have an afferent rather than an internuncial or efferent role. This agrees with the morphological work of Edinger (1906).

DISCUSSION

It was pointed out in the section on spontaneous activity that the resemblance between oscillatory electrical activity in the lancelet lamellae and the behaviour of a neural network model using digital computing techniques by Farley (1964) is so striking that it calls for further comment. Records from a loosely coupled isotropic network were much more similar to the lancelet records than those from other types of network. In the isotropic network waves of activity could spread equally in all directions. and by loosely coupled was meant that connexions existed that were long enough to reach back behind the trough of refractoriness that would otherwise limit the active area. Morphological information mainly from the work of Flood (1966, 1967) would justify both these requirements as far as the lamellae are concerned, in that they appear to be interconnected by many tight junctions, and the deep lamellae extend the full thickness of the myotome. If, as already suggested, the spontaneous activity is associated with tonus, then this system may be a very economical one, as Farley's network only requires the occasional injection of a stimulus to maintain diffuse oscillation. The occasional spikes in the nerve cord seen in Fig. 5*a* may be this adequate stimulus, and the larger extracellular muscle potentials may represent wave fronts in terms of Farley's experiments.

There can be no doubt that the slow and fast contractions demonstrated by Jansen

et al. (1963) in the muscle fibres of the hagfish *Myxine* are similar to those we have observed in the lancelet. The small and large potentials which accompany these contractions are also similar, except that their amplitudes are larger in the hagfish, presumably in association with the higher resting potentials. Furthermore, we have not observed the polarization plateau that repetitive stimulation produces in the slow fibres of the hagfish. Fast spikes are rather slower in the hagfish (10–15 msec. against 7 msec.) and both slow and fast electrical responses had a recovery phase of 50–100 msec. which did not occur in Amphioxus. The tench deep musculature provided similar fast spikes to those of the lancelet (Barets, 1961). The graded slow response that occurs in teleosts like the catfish (Barets, 1961) presumably arose at a post-cyclostome phase of evolution. Muscle-tail rates of conduction of 0·25 m./sec. in the lancelet compare with 1·1 m./sec. in *Cottus* (Hudson, 1969) and 1·6 m./sec. in frog muscle (Eccles, Katz & Kuffler, 1941).

The fact that the muscle lamellae of the lancelet are like those of *Ascaris* in having a high level of rhythmic spontaneous activity has been mentioned earlier. Some further points of similarity are that, as in the lancelet, spikes evoked by nerve-cord stimulation are of much shorter duration than spontaneous potentials, and shocks of high intensity may produce more than one spike. As many as three spikes may originate in this way in the nematode, but they arise from a depolarization plateau which is not the case in Amphioxus. Large spikes in *Ascaris* are followed by a gradual repolarization phase not found in the lancelet or the tench, but these spikes do decline in amplitude with distance from the nerve cord and we found evidence for this in the lancelet. However, we did not observe the prolonged refractoriness of up to two seconds demonstrated by deBell *et al.* (1963) in the nematode muscles.

Attempts to equate the superficial lamellae with the slow twitch by examining mechanical responses from a myotome from which the outer layer had been removed gave ambiguous results, and this question must be regarded as open despite evidence supporting this correlation.

Recordings from the spinal cord during slow swimming (unpublished data) do suggest that the Rhode cell system is involved in slow swimming as well as in large fast contractions (Guthrie, 1967). The stimulus frequencies for the onset of summation in the slow and fast system (2 Hz and 15 Hz) accord very well with the frequency of contractions in slow-swimming and fast-swimming lancelets examined by cinephotography; at the same time the slow twitch system appears too weak to flex the body through 90° as occurs during slow swimming.

A final point of comparison lies with the echinoderms. Cobb & Laverack (1966) were able to produce separate slow and fast contractions from the lantern retractor muscle of *Echinus* by local stimulation of the nervous system. The slow contraction was described by these authors as a delayed response. In *Astropecten* muscle fibres with muscle tails were found.

The results discussed in this paper help to reinforce the belief that in the lancelet we see a remarkable mosaic of vertebrate and invertebrate features.

SUMMARY

1. Two basic types of mechanical response can be elicited from the lancelet myotome by stimulation of the nerve cord by single shocks: a fast twitch with a rise time of 70–80 msec., and a slow twitch with a rise time of 200–250 msec.

2. The two twitch components can easily be elicited together to produce a biphasic contraction. A second type of biphasic contraction occurs more rarely in which the slow phase has a faster rise time and a higher peak tension.

3. The mean of all resting potentials was 48·94 mV. ± 15·5 mV. The commonest values were near 50 mV. and this must refer to the deep lamellae which form the bulk of the myotome.

4. Spontaneous potentials are a striking feature of the lancelet muscles.

5. The fast twitch is accompanied by an all-or-nothing, spike-like potential of between 30 and 70 mV. The slow twitch is accompanied by one or more potentials of between 1 and 10 mV. The type-2 biphasic mechanical response mentioned above is accompanied by two large potentials which attain similar amplitude, but differ in that the second is a graded response.

6. Some of the features of lancelet myotomal muscle have also been described in cyclostomes, nematodes and echinoderms.

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