J. Exp. Biol. (1969), **51**, 151–169
With 12 *text-figures*
Printed in Great Britain

SENSORY AND MOTOR INTERACTION IN THE LOCOMOTOR REFLEXES OF CRABS

BY W. H. EVOY* AND M. J. COHEN
Department of Biology, University of Oregon, Eugene, U.S.A.

(*Received* 19 *November* 1968)

INTRODUCTION

A variety of proprioceptive organs in the limb joints of Crustacea provide information about phasic and tonic aspects of joint action. These receptors consist primarily of stretch-sensitive sensory neurones coupled to elastic structures which are distorted by changes in joint position (Cohen & Dijkgraaf, 1961; Pringle, 1961). In many decapod Crustacea two different kinds of sense organs provide information about the joint between the meropodite and the carpopodite (*MC* joint) (Wiersma & Boettiger, 1959; Cohen, 1963*a*; Bush, 1965*a*). One type, the myochordotonal organ, consists of two groups of sense cells attached to a specialized receptor muscle, the accessory flexor (Barth, 1934; Cohen, 1963*a*). The receptor muscle is attached to the main flexor of the *MC* joint so that movement of the joint causes length changes in the muscle which in turn affect the attached sense cells. The sensory neurones can provide information about absolute joint position as well as the direction and rate of movement of the *MC* joint (Cohen, 1963*a*; Hwang, 1961). In the crab *Cancer magister* Dana the receptor muscle is innervated by a thick excitor axon and a thin inhibitor axon (Cohen, 1963*b*; Cohen & Hess, 1967; Dorai Raj & Cohen, 1964). Stimulation of the excitor axon causes contraction of the receptor muscle. This can modify the discharge rate of the sensory neurones independent of *MC* joint action. These sense organs are therefore directly under the influence of the central nervous system.

The second type of sense organ signalling information about the *MC* joint consists of sense cells located in elastic strands which span the joint. This type of sense organ was first described by Burke (1954) in the joint between the propodite and dactylopodite (*PD* joint). Subsequent studies by Alexandrowicz & Whitear (1957) and Whitear (1962) have shown one or more of these receptors to be present in all major joints of walking legs in Crustacea. Whitear (1962) describes this type of crustacean sense organ as similar to the insect chordotonal organ and calls it a chordotonal organ in Crustacea as well. The chordotonal organs are distinguished from the myochordotonal organs by being located at the joint whose action they detect. In addition, evidence will be presented here to show that specialized receptor muscles do not play a role in the function of the chordotonal organ receptors. Therefore their output is not capable of being modified by the central nervous system.

Proprioceptive reflexes in Crustacea appear to involve both excitatory and inhibitory influences at neuromuscular junctions. Bush (1962*a*, *b*; 1963) and Eckert (1959) have shown that in the peripheral segments of the pereiopods of *Carcinus* and other deca-

* Present address: Laboratory for Quantitative Biology, Department of Biology, University of Miami, Coral Gables, Florida 33124.

pods passive movement of these segments evokes reflexes which tend to resist the movement. In *Carcinus* Bush (1965*b*) described the reflexes evoked in the efferent nerves to the flexor and extensor muscles of the *MC* joint by passive movements of the joint. The responses consisted of reflexes that resist movement by the excitation of muscles antagonistic to the direction of movement.

The apparent redundancy of proprioceptive information provided by the myochordotonal and *MC* organs, combined with the presence of an independent muscle system for adjustment of the receptors, suggests a specialized role for the myochordotonal organs. We therefore proceeded to determine the way in which these two types of sense organs interact to control posture and walking in the crab. We recorded from the efferent neurones to the flexor and extensor muscles of the *MC* joint while manipulating the various proprioceptive organs activated by this joint. We also recorded from the efferent neurones to the receptor muscle of the myochordotonal organ to determine how the central control of this system might be involved in posture and locomotion.

MATERIALS AND METHODS

Experiments were carried out on mature male crabs, *Cancer magister* Dana, obtained from commercial market sources on the Oregon coast. After removal of the chelae and in some cases several of the walking legs, by autotomy if possible, the preparation was clamped ventral side up in a bath of aerated sea water at 10–12° C. The level of the bath was controlled to cover the openings to the gill chamber but not the walking legs. The posterior cuticle was removed from the meropodite of one or more walking legs. The legs used for recording were firmly clamped at the meropodite to allow free movement of the *MC* joint. The main body of the flexor muscle was then dissected free from the cuticle at the proximal end of the meropodite and laid back to expose the leg nerve. The exposed tissue was periodically bathed with cold physiological saline during the course of the experiment. A modification of Smith's (1947) *Cancer* saline was used, with the potassium ion concentration lowered to about $\frac{1}{3}$ the given value to conform with values determined by flame photometry of the body fluid.

The efferent innervation of the muscles of the *MC* joint was examined by supravitam methylene blue staining. For electrical recording the branches to the appropriate muscle were separated from the main leg nerve, cut distally and placed on platinum wire electrodes. Fine electrodes for recording from small nerve branches were made by electrolytically etching no. 28 platinum wire in a solution of 50% NaCN in 30% NaOH (Wolbarsht, MacNichol & Wagner, 1960). The nerves and recording electrodes were lifted into a pool of paraffin oil in the meropodite. In some cases it was necessary to use an aspirator to remove body fluid which seeped into the meropodite.

The experimental set-up is shown in Fig. 1. Mechanical stimulation of the *MC* joint proprioceptors was carried out by means of a device which consisted of a reversible electric motor geared down to pull on an arm by means of a speedometer cable and a series of ball-bearing mounted linkages. A potentiometer in a Wheatstone bridge circuit was also linked to the drive mechanism to provide a d.c. record of movement displayed on one beam of a four-beam Tektronix type 532 oscilloscope. The mechanism was attached to a pin driven through the carpopodite for flexion and extension of the joint. This enabled the *MC* joint to be moved at different reproducible rates over precise arcs.

Separate stimulation of the myochordotonal organs was accomplished with forceps mounted in a micrometer manipulator. The forceps were attached to the tendon of the accessory flexor muscle midway between its two heads. A linear potentiometer and bridge circuit registered the movement of this system on a second beam of the oscilloscope. The device was always attached to the accessory flexor tendon at the natural rest position of the joint. The tendon was then cut distal to the forceps to provide a reference indication of the rest position.

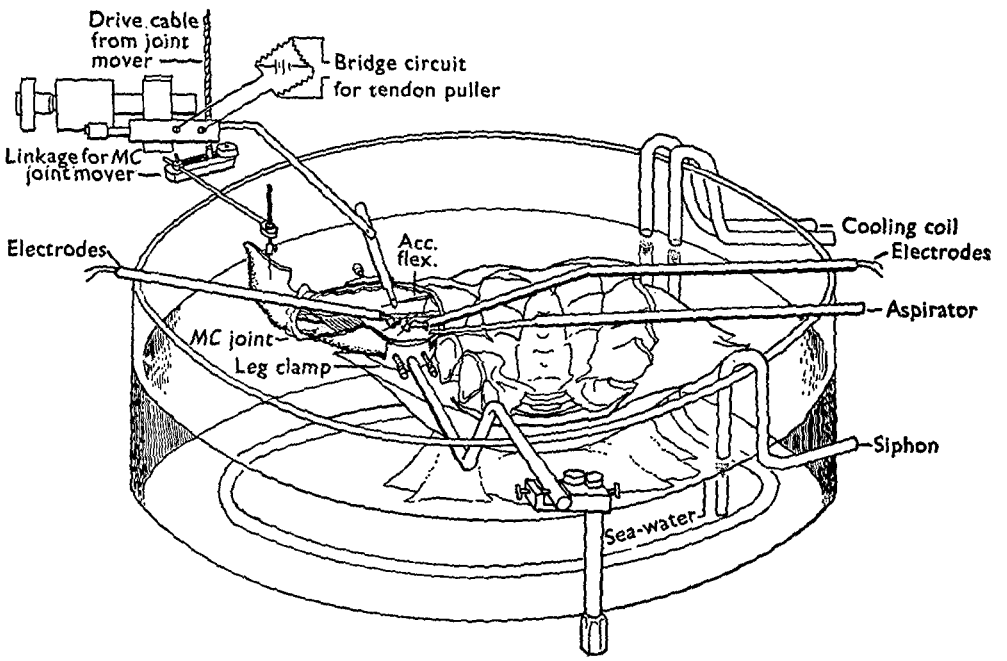

Fig. 1. Diagram of the experimental arrangement for simultaneous recording from extensor and flexor efferent neurones of the first right walking leg. The *MC* and myochordotonal organs can be stimulated separately. The *MC* organs are stimulated when the joint is moved by means of a motorized system of which only the final links are shown. The tendon of the accessory flexor (Acc. flex.) is cut and held in the forceps attached to a micrometer drive on the tendon puller. The crab is shown with its ventral surface upwards. All walking legs but the first right are removed.

For most of the experiments simultaneous recordings were made of the activity in two different nerves. These signals were led into Argonaut LRA-042 differential preamplifiers and displayed on the remaining two beams of the oscilloscope. In many cases this recording system did not provide sufficient amplification for photographic purposes, so the outputs from the preamplifiers were first led into a Tektronix type 502 oscilloscope and from the cathode follower output of that oscilloscope into the four-beam unit. The first oscilloscope then served as a monitor for visual observations and the second was used for recording with a Grass Kymograph camera.

Muscle fibres were penetrated with 3M KCl-filled glass microelectrodes, connected to a preamplifier with neutralized input capacitance.

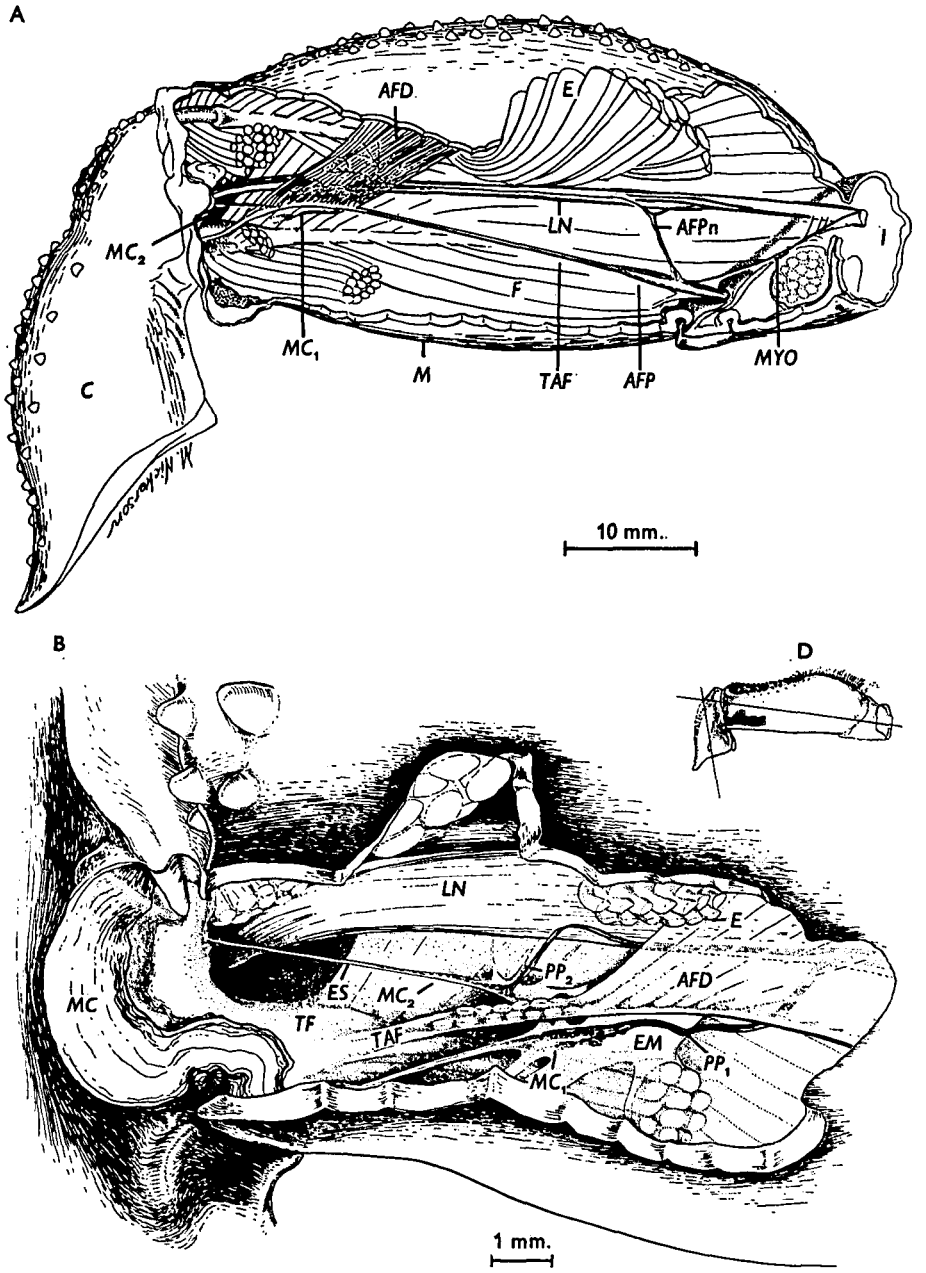

Fig. 2. A. Anterior view of the ischiopodite (I), meropodite (M) and carpopodite (C) of a right walking leg. The anterior cuticle is removed to show the proprioceptive systems and the leg musculature. *AFD*, distal head of accessory flexor; *AFPn*, branch of efferent nerves to proximal head; *AFP*, proximal head of accessory flexor muscle; *E*, extensor muscle; *F*, flexor muscle; MC_1, MC_2, chordotonal organs of *MC* joint; *TAF*, tendon of accessory flexor; *MYO*, sense cells of main myochordotonal organ; *LN*, leg nerve. B. Distal end of meropodite of a right walking leg with anterior cuticle removed to show the *MC* organs. The leg is oriented as if the animal were standing on the substrate as shown in the inset. *AFD*, distal head of accessory flexor muscle; *D*, dorsal side of leg; *E*, cut ends of extensor muscle fibres; *EM*, elastic membrane of MC_1; *LN*, leg nerve; MC_1, sensory cell bodies of MC_1 organ; MC_2, sensory cell bodies of MC_2 organ; PP_1, proximal processes of MC_1; PP_2, proximal processes of MC_2; *ES*, elastic strand of MC_2; *TAF*, tendon of accessory flexor muscle; *TF*, tendon of main flexor muscle; *MC*, *MC* joint.

RESULTS

Morphology and mechanical considerations

Sense organs

Each of the four stretch-sensitive cell groups of the *MC* joint is associated with the tendon or attachments of the accessory flexor muscle. There are two myochordotonal organs, the main organ described by Cohen (1963*a*) and a proximal organ (Hwang, 1961; Cohen, 1965). Both are attached to the torpedo-shaped proximal head of the accessory flexor. The *MC* organs, MC_1 and MC_2, are attached at the extreme distal end of the accessory flexor tendon, close to its insertion on the tendon of the main flexor (Fig. 2A, B).

Most of the tension which results from contraction of the accessory flexor muscle is due to the distal head, which pulls the tendon dorsally. When the distal head contracts, it pulls on the proximal head. This causes the proximal head to elongate just as it does during extension of the *MC* joint. This in turn excites the extension-sensitive receptor cells of the myochordotonal organ. Recordings from axons of the *MC* organs showed little if any response when the accessory flexor tendon was pulled in the direction that it moves during contraction of the distal head. The position of the *MC* organs at the rigid distal end of the tendon and the orientation of their endings in the connective tissue thus essentially isolate them mechanically from central control via efferent neurones to the accessory flexor muscle.

Efferent nerves

The efferent nerve supply to the muscles of the *MC* joint is shown in Fig. 3. The efferent fibres to the main flexor and extensor occur as distinct bundles of the main leg nerve and branch off at several points in the meropodite to supply the muscles. The main flexor nerve bundle lies just posterior to a large artery on the dorsal surface of the leg nerve. The extensor bundle lies just anterior to this artery. The main flexor nerve and all of its branches consist of five large fibres. In fresh whole mounts stained with methylene blue the fibres appear to be between 50 and 65 μ in diameter in the main bundles and between 10 and 15 μ in the initial branches. The three fibres of the main extensor bundle are between 25 and 50 μ in diameter and between 7 and 10 μ in the branches. The accessory flexor muscle is supplied by two fibres, a large one (about 50 μ in diameter) and a smaller one, about 10 μ (Cohen, 1963*a*, *b*). Separate branches from these two fibres innervate the proximal and distal heads of this muscle, as shown in Fig. 2A. These fibres only innervate the heads of the accessory flexor as far as can be determined by numerous dissections stained with methylene blue.

Combined reflexes evoked by both sensory systems

Reflexes to extensor and flexor

Responses from efferent nerves. The responses in the main flexor and extensor efferent nerve fibres to movements of the joint with both the *MC* and myochordotonal systems intact are classical resistance-stretch reflexes. In fresh preparations these responses were generally superimposed upon an endogenous background discharge in at least one fibre of the efferent nerve. At the normal rest position of the joint, which

is an angle of about 100° between the meropodite and carpopodite, the frequency of this background was typically between one and five impulses per second. A decrease in the frequency of this discharge was the first sign of an aging preparation.

Flexion of the joint from the rest position evoked a phasic burst in the extensor efferents and depression of activity in the flexor efferent (Fig. 4A). During maintained flexion there was often a lower frequency tonic discharge in the extensor efferent, although not to the extent seen in the flexor on maintained extension. Movement back to the rest position from the flexed position evoked a burst in the flexor efferents and often phasically inhibited those in the extensor.

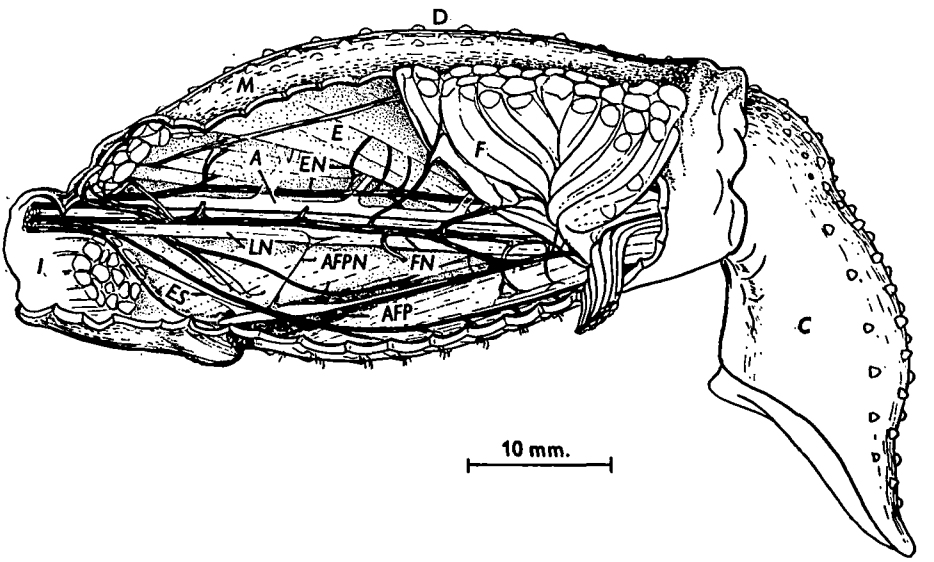

Fig. 3. Posterior view of meropodite of a right walking leg to show muscles and efferent nerves. The main flexor muscle is laid back to expose the nerves and the accessory flexor muscle. *A*, artery; *AFP*, proximal head of accessory flexor; *AFPN*, efferent nerves to proximal head of accessory flexor; *C*, carpopodite; *D*, dorsal edge of leg; *E*, extensor muscle; *EN*, efferent nerves to extensor; *ES*, elastic strand of main myochordotonal organ; *F*, main flexor muscle; *FN*, efferent nerves to main flexor; *I*, ischiopodite; *LN*, main leg nerve; *M*, meropodite.

Extension of the *MC* joint from the rest position evoked a burst in the flexor efferent and a simultaneous silencing of the background activity in the extensor efferent (Fig. 4B). Flexion of the *MC* joint from an extended position back to rest evoked a discharge in the extensor efferent and a simultaneous silencing of the flexor discharge. At the rest position the flexor discharge returned to the background level, often after a rebound from the inhibitory influence of flexion.

Intracellular recordings from muscle. Intracellular recordings from individual flexor muscle fibres (Fig. 5) show that the responses to passive joint movements are all mediated by excitatory nerve fibres. Summation of excitatory junctional potentials (ejp's) from several motor nerve fibres with endings on a single muscle fibre can be surmized from the complexity of the compound ejp's. Individual excitatory nerve fibres produced a considerable amount of neuromuscular facilitation at high frequencies and complex depolarizations up to 30 mV. in amplitude were noted in some muscle fibres. Occasionally, a burst of 5–10 mV. hyperpolarizing inhibitory

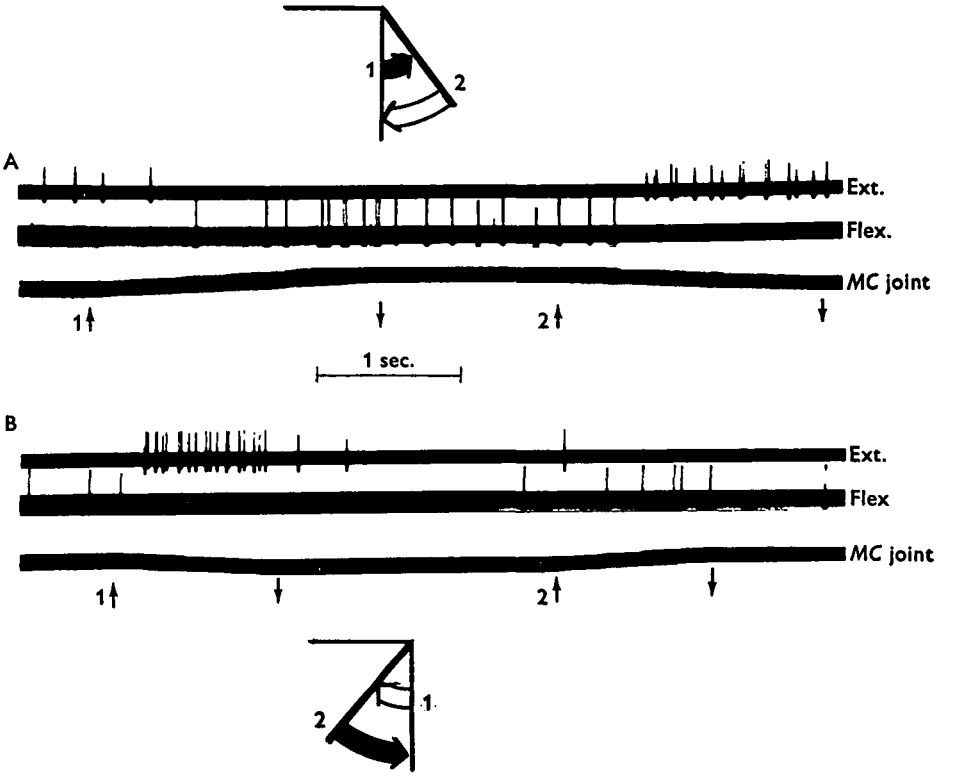

Fig. 4. Simultaneous recording from extensor and flexor efferent nerve fibres of the first right walking leg with the entire proprioceptive system intact. Top beam, extensor efferent; second beam, flexor efferent; lower beam, movement of *MC* joint. Upward movement of lower beam indicates extension; downward movement, flexion. Upward arrows indicate beginning of movement; downward arrows indicate cessation of movement. A. (1) Extension of *MC* joint from rest position, and (2) flexion back to rest. B. (1) Flexion of *MC* joint from rest, and (2) extension back to rest. Note the reciprocal responses in the extensor and flexor motor neurones that form the basis of the resistance reflexes.

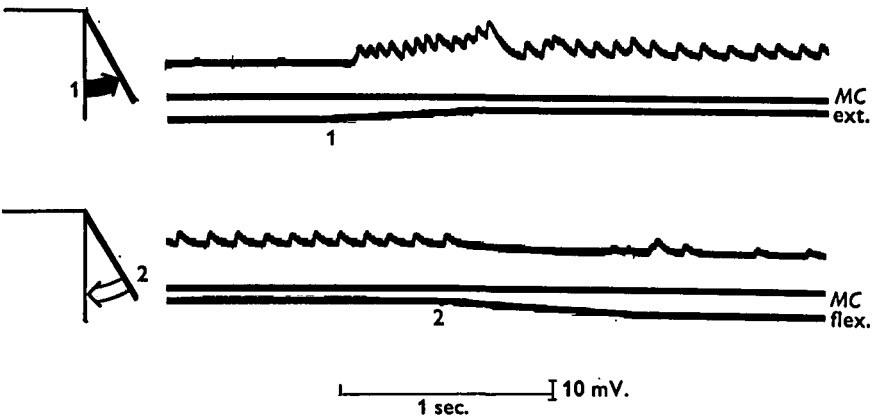

Fig. 5. Intracellular responses of a single main flexor muscle fibre to movements of the *MC* joint with the entire proprioceptive system intact. Top beam, flexor fibre; bottom beam, movements of the *MC* joint of the first right walking leg. Upward movement of the bottom beam indicates extension of the joint from its rest position; downward movement indicates flexion. Middle beam serves as a reference point to aid in detecting deflexion of the lower beam. This fibre had a 68 mV. resting potential. (1) Extension of joint from rest; (2) flexion back to rest. Note the increase in junctional potentials when the joint is extended and the decrease in the lower record when the joint is flexed.

junctional potentials (ijp's) appeared in extensor and flexor muscle fibres. However, these could not be reliably evoked by passive joint movements, nor was any other sensory input found which consistently produced activity in the peripheral inhibitors.

Reflexes to receptor muscle

Simultaneous monitoring of the reflex activity in efferents to the main and accessory flexors permitted a comparison of the effects of the different proprioceptive inputs on these two muscles. Under the influence of both the *MC* organs and the myochordo-

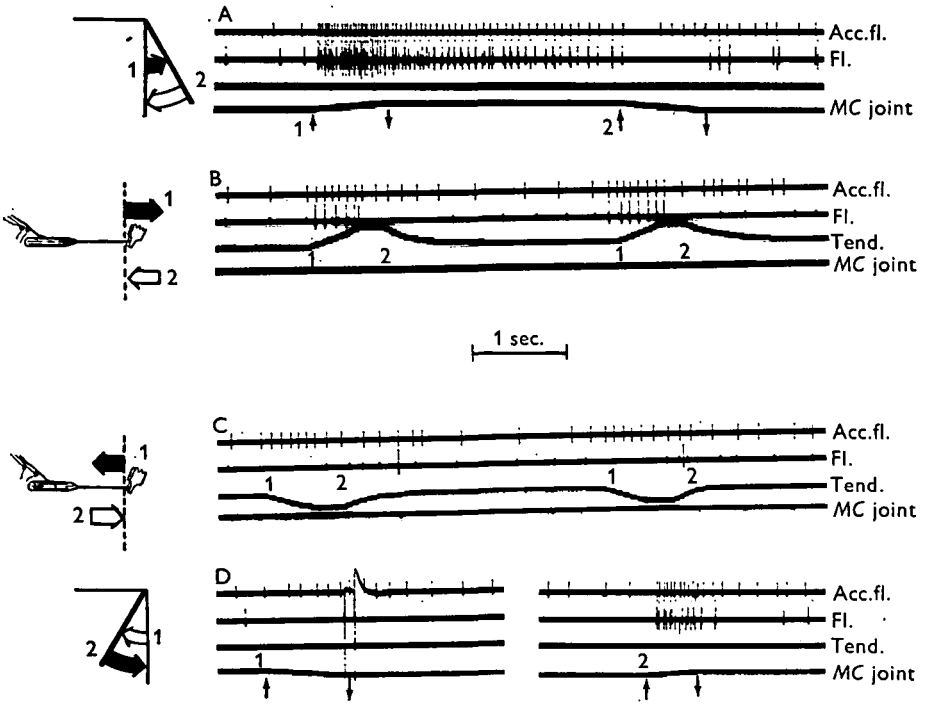

Fig. 6. Simultaneous recording from accessory and main flexor efferent neurones of first right walking leg. These illustrate the different reflex effects of the myochordotonal and *MC* sensory systems. Top beam, accessory flexor efferent; next to top, main flexor efferent; next to bottom, movement of accessory flexor tendon; bottom, movement of *MC* joint. Upward movement of the lower beams indicates extension, downward indicates flexion. A. (1) The *MC* joint is extended from rest with the whole proprioceptive system intact; (2) flexion back to rest. B. (1) The accessory flexor tendon is pulled from its rest position as in joint extension to show influence of the myochordotonal organ alone; (2) release of the tendon back to its rest position to mimic flexion. The *MC* joint is held at the rest position. C. (1) Release of the tendon from rest to mimic flexion; with the *MC* joint held at the rest position; (2) tendon pulled back to rest position. D. (1) Flexion of the *MC* joint from rest with the entire proprioceptive system intact; (2) extension back to rest. The *MC* joint is held stationary while the tendon is pulled. Upward arrows indicate the beginning of movement; downward arrows cessation of movement.

tonal organs, the reflex control of the receptor muscle appears generally to parallel that of the main flexor. Activity in the accessory flexor efferent nerve was picked up from the branch to the proximal head of that muscle. Simultaneous recording from the branches to the proximal and distal heads verified that both share a common

excitatory axon. With all of the proprioceptive organs intact, both the accessory and main flexors were excited by extension of the joint and centrally inhibited by flexion back to rest (Fig. 6A). A very slowly decreasing 'tonic' discharge was seen on maintained extension.

Flexion from the rest position, again with all the proprioceptive organs intact (Fig. 6D), evoked a variable response in the accessory flexor and depression of background discharge in the main flexor. During maintained flexion tonic discharge in the accessory flexor efferent may be depressed slightly, may not be altered, or may show a slight increase relative to the background activity of the rest position. In no case was the response to flexion of the same magnitude as that to extension. Extension from the fully flexed position back to rest caused bursts in both accessory and main flexor efferents.

Separation of myochordotonal and MC organ reflexes

MC organ reflexes. In order to distinguish the reflexes evoked by the myochordotonal organ from those initiated by the *MC* organs, the tendon of the accessory flexor muscle was cut midway between the two heads. The responses evoked only by *MC* organ input were observed by fixing the proximal stump of the tendon to hold the myochordotonal organ at its rest position and then moving the *MC* joint. Under these conditions the reflexes evoked by the *MC* organs appeared similar to those caused by the combined input of both systems. However, the element of variability in the receptor muscle response, particularly on flexion from rest, was greatly reduced. With only the *MC* organs activated, the accessory flexor background was now generally silenced on flexion of the joint from the rest position.

Myochordotonal organ reflexes. The reflexes evoked by the myochordotonal organ alone were examined by fixing the *MC* joint and moving the tendon attached to the proximal head of the receptor muscle. Pulling the accessory flexor tendon distally from its rest position causes a response in the myochordotonal organs that mimics the effect either of joint extension or a shortening of the distal head of the accessory flexor (Fig. 6A, B). The discharge frequency in both accessory and main flexor efferents is increased although the response is far weaker than that to joint movements with either the whole proprioceptive system intact or the *MC* organs alone. At the same time, the discharge frequency in the extensor efferent neurones is usually decreased (Fig. 7B). Return of the stretched myochordotonal system to the rest position caused a drop in discharge frequency of the main flexor and sometimes of the accessory flexor efferent neurones (Fig. 6B). Usually, the discharge in the flexor efferents is completely silenced, whereas the activity in the accessory flexor efferent often gradually decreases to the background level but is not completely suppressed. The frequency in the extensor neurones always increases under these conditions (Fig. 7B).

The opposite signs of the reflex response in the efferent fibres to the main flexor and to the receptor muscle (accessory flexor) substantiate the anatomical findings that separate neurones innervate these two muscles. The separate innervation of these two muscles is further substantiated by the comparison of simultaneous recordings from their efferent neurones. There is no temporal correspondence in the discharge of motor fibres innervating the two separate muscles (Fig. 6).

Movement of the joint with the tendon of the proximal head held stationary evoked responses which were not detectably different from the responses with the whole proprioceptive system intact. This is because the reflexes evoked by the *MC* organs during movement dominate those caused by the myochordotonal organ so that the effects of the latter system are not readily observable when recording with both systems intact. The *MC* organs evoke simple reciprocal reflex responses to the antagonistic

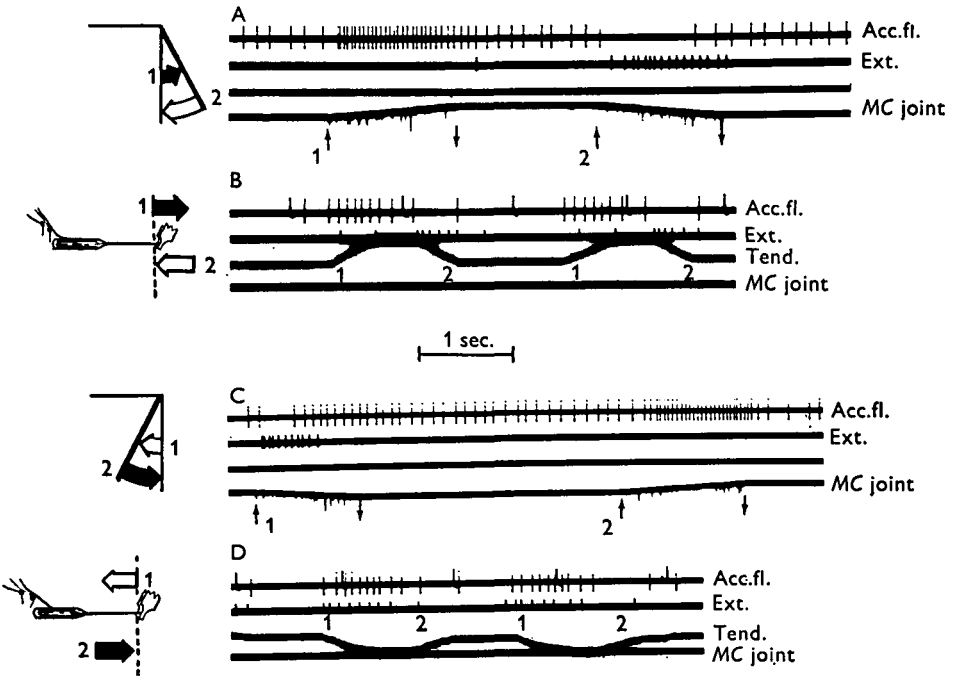

Fig. 7. Simultaneous recording from accessory flexor and extensor efferent neurones of first right walking leg. Top beam, accessory flexor efferent; next to top, extensor efferent; next to bottom, movement of accessory flexor tendon; bottom, movement of *MC* joint. Upward movement of the last two beams indicates extension; downward movement flexion. Upward arrows indicate beginning of movement; downward arrows cessation of movement. A. (1) Extension of *MC* joint from rest with the entire proprioceptive system intact; (2) flexion back to rest. B. (1) the accessory flexor tendon is pulled from rest as it would be during joint extension; (2) the tendon is returned to rest position. C. (1) Flexion of *MC* joint from rest with the proprioceptive system intact; (2) extension back to rest. D. (1) Release of the accessory flexor tendon from rest to mimic joint flexion; (2) the tendon is returned to rest position. The *MC* joint is held in the rest position during all of the tendon pulls.

muscle groups. The effect is to resist any imposed movement of the *MC* joint. The myochordotonal input, by contrast, reinforces the *MC* organs in their effect on the two main muscle groups, but has a quite different feedback relationship to its own receptor muscle. *Movements of the myochordotonal system in either direction away from its natural rest position excite the receptor muscle. Movements back toward the rest position decrease the discharge frequency in the excitatory neurone to the receptor muscle.*

Tonic and phasic reflex responses

Recordings from efferent fibres. The 'tonic', or slowly adapting frequency of firing in the efferent nerves of the main and accessory flexors is related to the degree of

extension from the rest position (Fig. 8). Approximately equal steps of extension produced bursts, with recruitment of purely 'phasic' units on each movement and an almost constant frequency of firing in one or more units at a given maintained position. The frequency of 'tonic' discharge at the fully extended position was three to four times as great as that of the background discharge in the rest position. The 'tonic' frequencies at intermediate steps between rest and full extension were proportional to the degree of imposed extension.

The frequency of the 'phasic' response was quite constant for a given rate of movement over most of the range of extension from rest. During movements of the joint the 'phasic' response generally appeared to include all the excitatory fibres in an efferent nerve.

Intracellular recordings from muscle. Intracellular recordings of the reflex responses in flexor muscle fibres indicate the relative effectiveness of the 'phasic' and 'tonic' resistance to position change of the *MC* joint (Fig. 9). During the movement there is

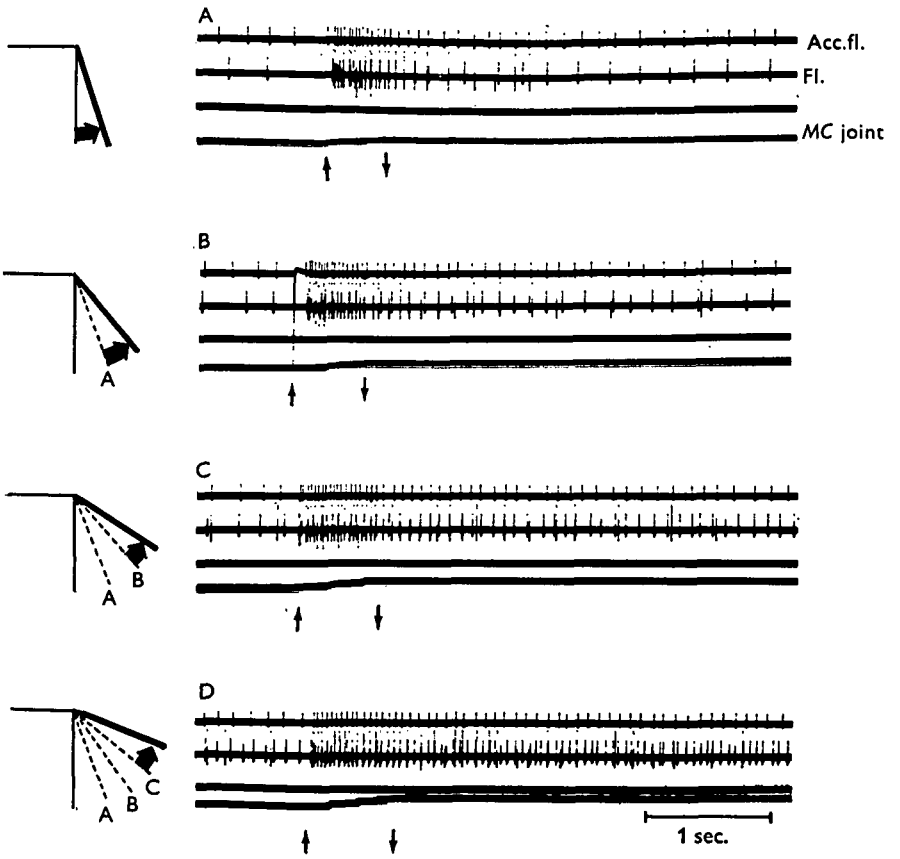

Fig. 8. Simultaneous recording from accessory flexor and main flexor efferent neurones of the first right walking leg. The *MC* joint is moved in steps with the entire proprioceptive system intact. Top beam, accessory flexor efferent; next to top, main flexor efferent; bottom beam, movement of *MC* joint. Upward movement of the bottom beam indicates extension. In A the initial position of the bottom beam indicates the rest position of the joint. A–D show successive steps of extension and the corresponding maintained discharge at each position. Upward arrows indicate beginning of movement; downward arrows cessation of movement.

marked facilitation of junctional potentials, accompanied by recruitment of additional units. This results in a maintained plateau of depolarization, and therefore presumably of tension, in muscles which oppose the movement. Following the movement, but at

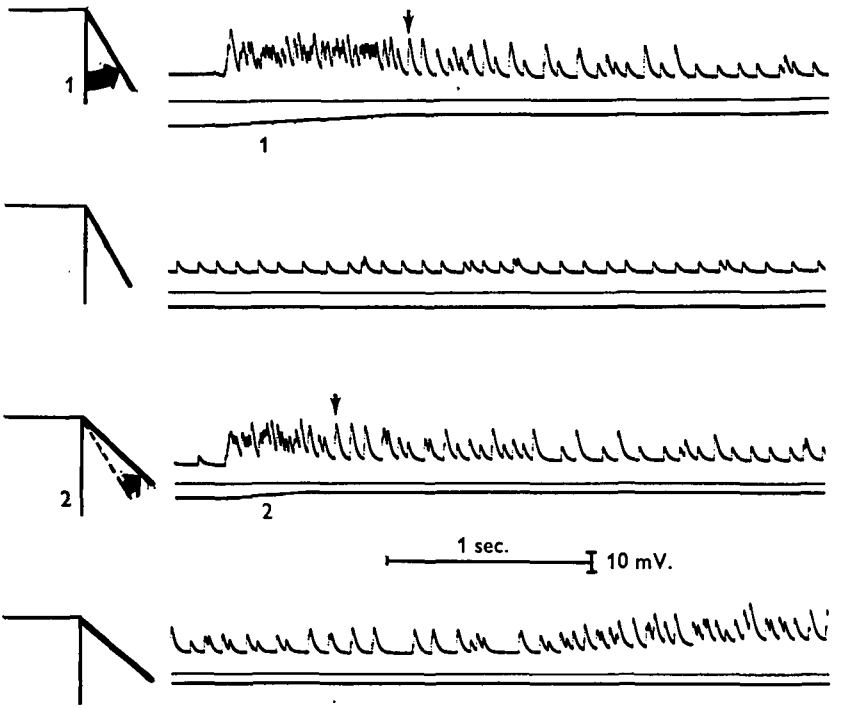

Fig. 9. Intracellular responses of single flexor muscle fibre to movements of the *MC* joint. The entire proprioceptive system is intact. Top beam, flexor muscle fibre; bottom beam movement of the *MC* joint in the first right walking leg. Upward deflexion of the bottom beam indicates extension of the joint from its rest position; downward deflexion indicates flexion. The fibre has a 56 mV. resting potential. Extension from rest is in two steps. Note the greater occurrence of summating junctional potentials during maintained extension after movement 2. Arrows indicate summating potentials.

positions away from rest, there is a steady arrival of ejp's whose frequency is proportional to the degree of maintained extension (cf. Figs. 8, 9). Toward the limits of maintained extension, units which were present only in the 'phasic' response at less extended positions continue to add components of depolarization during the 'tonic' phase. In Fig. 9 the secondary depolarizations indicated by the arrows in a few cases did not always arise at a constant level of depolarization. This indicates that they are junctional potentials produced by nearly coincident arrival of motor impulses rather than actively conducted muscle spikes. Thus, there is a definite positional sensitivity of the resistance reflex to *maintained* joint deflexions which is accentuated at the limits of joint movement.

Reflex interactions between legs

In order to examine the interaction of proprioceptive influences in one leg with efferent activity in others, experiments were performed involving mechanical stimulation of a leg while recording simultaneously from that leg and others. Considerable spread of both *MC* and myochordotonal inputs was seen between ipsilateral legs, and much less between contralateral legs. The reflex responses were, however, always stronger in the stimulated leg than in the other (Fig. 10). The spreading influence of these inputs to other legs maintained the general nature of the resistance reflexes, i.e. extension produced flexor activity in both legs. However, these responses were never as precise in the unstimulated leg and did not show the extent of positional sensitivity noted above. Repeated movements of one leg always produced resistance reflexes in that leg long after the responses had disappeared in an unstimulated leg. After several minutes rest the reflexes would reappear in the unstimulated leg. Cutting the leg nerve at the basi-ischial joint of the stimulated leg abolished all resistance reflexes normally spreading to other legs, thus controlling for possible mechanical disturbance of receptors in other legs.

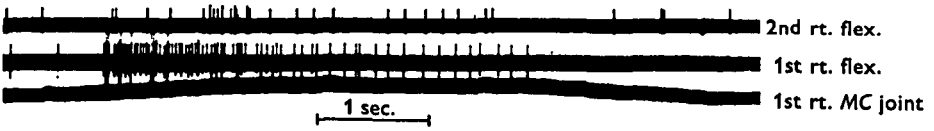

Fig. 10. Simultaneous recordings from flexor efferent neurones of two different walking legs. Upward movement of the lower beam indicates extension of the *MC* joint in the first right leg; downward, flexion. Top beam, efferent neurone to flexor of second right walking leg; middle beam, efferent neurone to flexor of first right walking leg.

To determine whether removal of most of the walking legs causes a significant reduction in excitability of the nervous system, a few control experiments were carried out with all of the walking legs intact. The last three pairs of walking legs were bound together to prevent them from disturbing the stimulating and recording apparatus. There was no detectable difference in the nature or intensity of any response evoked in the same or another leg by mechanical stimulation.

DISCUSSION

Reflex control of the muscles in the meropodite of the walking legs of *Cancer* is influenced by two receptor systems, the myochordotonal organ and the *MC* organs. Both sensory groups act on the flexor and extensor muscles of the *MC* joint to produce reflexes which resist movement of the joint. It would appear from this that the two sensory structures play duplicate roles and are therefore redundant. However, the myochordotonal organ is under efferent control from the central nervous system, while the *MC* organs are independent of any central influence. This, together with the distinctly different reflex effects that the two sensory systems have on the receptor muscle, leads us to postulate a unique role played by the myochordotonal organ in the control of posture and locomotion.

A tentative scheme of connexions between the sensory input from both proprio-

ceptive systems and the motor output can be drawn from the observations on evoked reflexes. In the diagram shown in Fig. 11, input from flexion-sensitive units of the *MC* organs and the myochordotonal organ excites the extensor motor units and centrally inhibits the flexors. Extension-sensitive units of these two types of proprioceptive organs have the reciprocal effect; they inhibit the extensor units and excite the flexors.

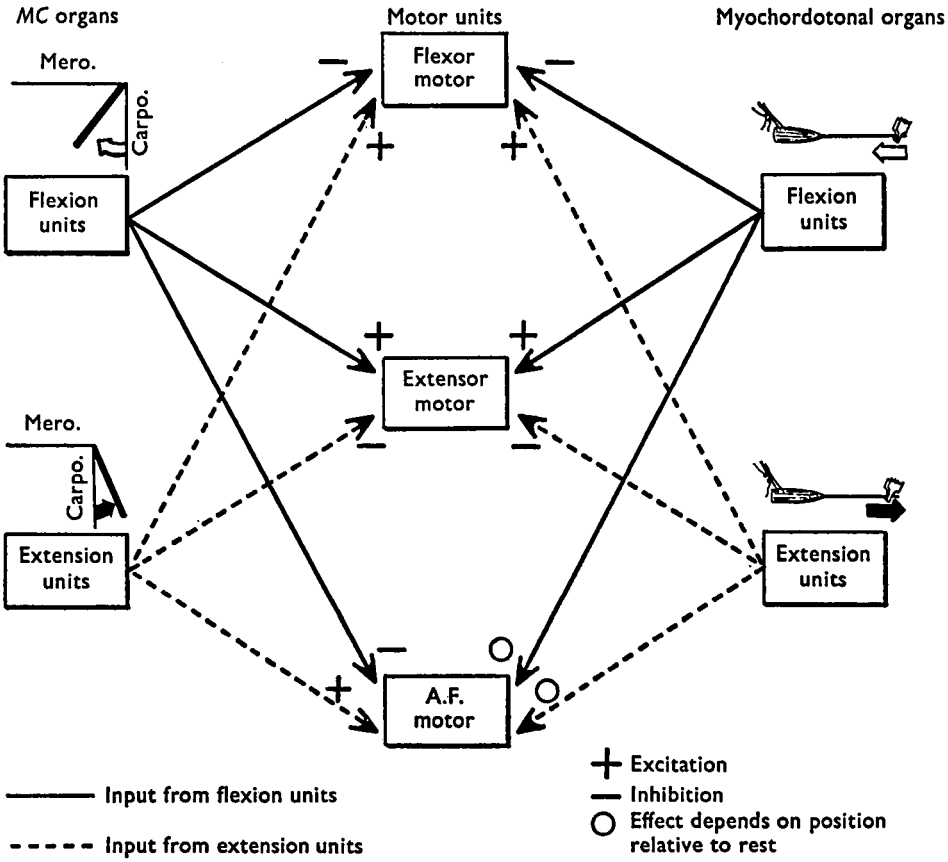

Fig. 11. Summary of probable proprioceptor connexions with motor units in the thoracic ganglion which control the muscles of the *MC* joint. Solid arrows indicate extension; open arrows flexion. Extension-sensitive units of the *MC* organs excite the flexor motor neurones and inhibit the extensor motor neurones. Flexion-sensitive units excite the extensor motor neurones and inhibit the flexor motor neurones. The effect of *MC* organ input on the single motor unit of the accessory flexor is like that on the main flexor muscle. The myochordotonal input to the main flexor and extensor acts much like the *MC* organ input. The myochordotonal effect on the accessory flexor system is bidirectional; the input from the myochordotonal organ tends to excite the accessory flexor whenever the joint is moved away from the rest position.

The effect of the two sensory systems on the main moving muscles of the *MC* joint is therefore one of mutual reinforcement. These connexions are clearly responsible for the resistance reflexes which tend to counteract movement of the *MC* joint and are in accord with similar resistance reflexes described by Bush (1965*b*) in *Carcinus*.

The connexions of the two sets of proprioceptive organs to the single motor neurone of the accessory flexor are more complex. The *MC* organs act on the receptor muscle just as if it were a flexor muscle involved in resistance reflexes, i.e. extension of

the joint excites the muscle, while flexion causes a decrease in the activity of the excitatory axon to the muscle (Fig. 11). Feedback from the myochordotonal organs to the receptor muscle is quite different. Movement of the accessory flexor in the direction of either flexion or extension from its natural resting position results in increased activity in the motor fibre to the accessory flexor. Movement toward the rest position causes a drop in the discharge frequency in the motor axon. The drop in frequency with a return toward the rest position may be either a gradual decrease toward the background level, or it may be an abrupt silencing of all activity. *Therefore the principal effect of the myochordotonal input feed-back to its own receptor muscle is to signal deviation of that muscle from its resting length* and to evoke reflexes that restore the muscle to this length (Fig. 12).

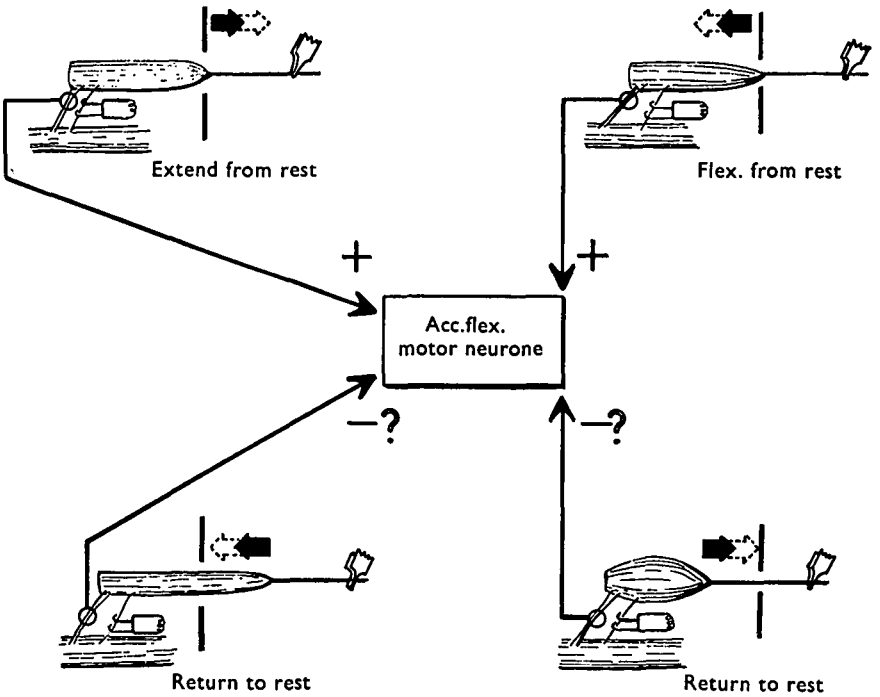

Fig. 12. Summary diagram of probable connexions of myochordotonal system to accessory flexor motor neurone. The heavy vertical line in each diagram indicates the rest position of the proximal head of the accessory flexor muscle. Solid arrows indicate movements of the tendon that mimic extension of the joint; open arrows mimic flexion. In the top two diagrams movements away from the rest position in either direction excite the accessory flexor motor neurone. Return of the system to rest in the bottom diagram has an indeterminate effect. In some cases the system appears to be centrally inhibited, in others there is a passive return to the background frequency of the rest position.

Factors which determine the posture of the rest position

The position of the *MC* joint is dynamically determined by the combined input of two receptor systems acting through resistance reflexes. These resistance reflexes have a stereotyped predictable component from the *MC* organs which is determined only by the position of the joint. The reflexes evoked by the myochordotonal organ are affected by an additional variable, the discharge frequency in the motor neurone to the

receptor muscle. The reflexes evoked by the *MC* organs are strongest at the extremes of maintained joint position, i.e. either maximal flexion or extension. This suggests that these resistance reflexes tend to drive the *MC* joint to some position mid-way between the extremes. Once the joint is forced into the mid-region, it may be more precisely adjusted and locked into a rest position by the feedback loop of the myochordotonal organ to its own receptor muscle and to the main moving muscles.

Stabilization of joint rest position via efferent feedback to receptor

In order to discuss how the joint may be held fixed in a given rest position it is necessary to reconstruct what happens when the joint deviates slightly from this position. Assume that the joint is slightly extended from the rest position. The *MC* reflexes are weakest in this region as mentioned above, and we are therefore concerned primarily with reflexes evoked by the myochordotonal organ. When the joint is slightly extended, the input from the myochordotonal organ will excite the flexor and inhibit the extensor discharge, thus flexing the leg back toward the rest position. During the initial extension from rest the myochordotonal feedback to its receptor muscle will excite the muscle because the proximal head has been moved away from its rest position (see Fig. 12). This will tend to reinforce the drive on the flexor muscle because the distal receptor muscle head is excited and contracts to stretch the proximal head and sense cells as if extension of the *MC* joint has occurred. However, upon flexion back toward the rest position the discharge to the receptor muscle decreases. This relaxes the distal head and allows the proximal head of the receptor muscle to shorten passively due to the elasticity in the system. This reinforces the shortening caused by the flexion movement. The net effect is to accelerate the flexion-resistant reflexes evoked by the myochordotonal organ. This is achieved by exciting the extensor muscle to check the flexion before it moves very far past the rest position. As the joint drifts past rest into a slightly flexed position, the resistance reflexes of the myochordotonal organ excite the extensor muscle. This checks the flexion drift and begins to extend the joint back toward rest. In the slightly flexed position the feedback to the receptor muscle now excites the distal head to take up any slack caused by flexion and this stretches the proximal head until it reacts as if the joint is slightly extended. This then checks the extension by exciting the flexor muscle. Therefore we speculate that the effect of the efferent feedback to the receptor muscle is to *anticipate* where the joint will be before it actually gets there and to throw into play resistance reflexes to the movement that is actually taking place. This could tend to dampen oscillatory movements and terminate them closer and closer to the final rest position.

Re-setting the primary rest position of the joint

The data suggests that the efferent loop to the myochordotonal organ could be used to re-set the primary orientation of the *MC* joint. This could be useful in altering the attitudinal poise of the animal from one of rest to one of alert defense. The following hypothesis is proposed.

The key to altering the primary position of the *MC* joint is the discharge frequency in the efferent neurone to the receptor muscle. Increased discharge in the accessory flexor motor neurone causes shortening of the distal head, which results in pull on the proximal head and consequent discharge of the myochordotonal organ. This discharge

is identical with that which occurs when the *MC* joint is passively extended (Cohen, 1963*a*; Dorai Raj & Cohen, 1962). Three different factors can affect the discharge frequency in the receptor muscle motor neurone: (1) the discharge from the *MC* organs, (2) the discharge from the myochordotonal organ, and (3) a central component which is probably independent of the input from the joint receptors. The central drive on the receptor muscle motor neurone could conceivably be varied and thus alter the discharge frequency in the myochordotonal receptors independent of joint position. This in turn would alter the drive of the myochordotonal input on the flexor and extensor muscles via resistance reflexes. The final consequence would therefore be a shift in position of the *MC* joint.

Assume that the central drive on the receptor motor neurone suddenly increases when the joint is in a primary rest position. This causes contraction of the distal head and results in a pulling on the proximal structures, with concomitant sensory input from the myochordotonal system as if the joint were actually extended. The altered myochordotonal input resulting from stretching the proximal structures evokes resistant reflexes that excite the flexor and inhibit the extensor muscles (Fig. 11). The myochordotonal feedback to its own distal head increases the discharge frequency in the receptor efferent because the system is pulled away from its primary resting length (Fig. 12). This feedback reinforces the initial effect of the increased efferent discharge and supports the flexion brought about by the initial lengthening of the proximal head. Once flexion begins the proximal head is passively shortened and this reflexly reduces the frequency in the receptor muscle motor neurone because it is moving back toward its primary rest length (Fig. 12). The joint will continue to flex until the proximal head once again approaches its primary resting length and the myochordotonal reflex drive back to the receptor efferent is reduced to approach what it was in the primary resting position before the new central bias was exerted. The joint will then come to rest at some new position that is flexed from the primary rest position and in which the proximal head of the receptor muscle approaches the length that it had in its primary rest position. This new flexed position will be held as long as the increased central drive on the receptor efferent is maintained. The new position can be viewed as the change in joint position necessary to correct the change in receptor muscle length which was brought about by altering the discharge frequency in the motor neurone to the receptor muscle. Once the new rest position is achieved, it would be stabilized just as described for the primary rest position. A drop in the central drive on the receptor motor neurone would result in the joint assuming a position that is extended from the primary rest position by precisely the same factors coming into play but with reflexes of opposite sign. The system seeks to restore the proximal head of the receptor muscle to a fixed length characteristic of the rest position.

The model which has been constructed from observations of reflexes evoked by different components of the proprioceptive system suggests a mechanism for stabilization and adjustability of walking leg posture. Further experiments on freely moving animals in which the inputs from various components of this system were altered in controlled ways have corroborated and extended these ideas (M. J. Cohen and W. H. Evoy, in preparation). In another system, the dorsal abdominal muscle receptor organs of crayfish, it has been found that the muscle receptor acts as a peripheral reference mechanism for control of segment positions and that highly specific extra-

segmental central pathways exist for the alteration of the receptor signal (Fields, 1966; Fields, Evoy & Kennedy, 1967).

The muscle-spindle system of vertebrates also possess routes of excitation which are independent of those to extrafusal muscle fibres and thus can evoke secondary reflexes to the working muscles (Granit, Holmgren & Merton, 1955; Bessou, Emonet-Denand & Laporte, 1963; Leksell, 1945). This has led Eldred, Granit & Merton (1953) to postulate that the spindles play a role in the tonic setting of muscle length to determine basic posture in the vertebrates. There seems to have been a high degree of convergent evolution in vertebrates and Crustacea in the realm of the control of posture and locomotion. This indicates that studies on the relatively accessible crustacean central nervous system may provide information of general interest regarding the formulation of motor commands.

SUMMARY

1. Proprioceptive reflexes involved in control of the mero-carpopodite (*MC*) joint in walking legs of *Cancer magister* are mediated by four groups of sensory cells: (*a*) two cell groups (myochordotonal organs), located proximally in the leg segment, which are associated with a receptor muscle and come under efferent control, and (*b*) two cell groups (*MC* organs) located distally which signal joint position and movement but are independent of efferent control.

2. Resistance reflexes, which oppose passive movements of the joint, are evoked by both types of proprioceptive organs. Reflexes evoked by the *MC* organs are maximally effective at the extremes of joint position.

3. Reciprocal inhibition of antagonistic muscle groups appears to occur entirely at synapses within the central nervous system.

4. Efferent control of the receptor muscle comes under the influence of both the myochordotonal and *MC* organs. The reflexes evoked by the *MC* organs on the receptor muscles are similar to those which impinge on the main flexor. The myochordotonal organ excites the receptor muscle on joint movements away from the rest position. Interaction of input from the two types of sense organs provides the system with a dynamically determined reference point for the basic postural position. This may be altered by efferent control of the receptor muscle.

5. Reflexes evoked by both types of proprioceptive organ spread to the other walking legs, but are more labile than reflexes to the same leg.

Supported by U.S. Public Health Service Research Grant R01 NB 01624 and Career Development Grant 5-K3-NB-4992 to M.J.C. During the course of this work W.H.E. was supported by U.S. Public Health Service Physiology Training Grant 6665-TIGM 33605.

REFERENCES

ALEXANDROWICZ, H. L. & WHITEAR, M. (1957). Receptor elements in the coxal region of Decapod Crustacea. *J. mar. biol. Ass. U.K.* **36**, 603–28.

BARTH, G. (1934). Untersuchungen über Myochordotonalorgane bei dekapoden Crustaceen. *Z. wiss. Zool.* **145**, 576–624.

BESSOU, P., EMONET-DENAND, F. & LAPORTE, Y. (1963). Occurrence of intrafusal muscle fibre innervation by branches of slow motor fibres in the cat. *Nature, Lond.* **198**, 594–5.

BURKE, W. (1954). An organ for proprioception and vibration sense in *Carcinus maenas*. *J. exp. Biol.* **31**, 127–38.

BUSH, B. M. H. (1962*a*). Peripheral reflex inhibition in the claw of the crab, *Carcinus maenas*. *J. exp. Biol.* **39**, 71–88.

BUSH, B. M. H. (1962*b*). Proprioceptive reflexes in the legs of *Carcinus maenas*. *J. exp. Biol.* **39**, 89–106.

BUSH, B. M. H. (1963). A comparative study of certain limb reflexes in decapod crustaceans. *Comp. Biochem. Physiol.* **10**, 273–90.

BUSH, B. M. H. (1965*a*). Proprioception by chordotonal organs in the mero-carpopodite and carpo-propodite joints of *Carcinus maenas* legs. *Comp. Biochem. Physiol.* **14**, 185–99.

BUSH, B. M. H. (1965*b*). Leg reflexes from chordotonal organs in the crab, *Carcinus maenas*. *Comp. Biochem. Physiol.* **15**, 567–87.

COHEN, M. J. (1963*a*). The crustacean myochordotonal organ as a proprioceptive system. *Comp. Biochem. Physiol.* **8**, 223–43.

COHEN, M. J. (1963*b*). Muscle fibres and efferent nerves in a crustacean receptor muscle. *Q. Jl Microsc. Sci.* **104**, 551–9.

COHEN, M. J. (1965). The dual role of sensory systems: detection and setting central excitability. *Cold Spring Harb. Symp. quant. Biol.* **30**, 587–99.

COHEN, M. J. & DIJKGRAAF, S. (1961). Mechanoreceptors. In *Physiology of Crustacea*, vol. 2, pp. 65–108. New York: The Academic Press.

COHEN, M. J. & HESS, A. (1967). Fine structural differences in 'fast' and 'slow' muscle fibers of the crab. *Am. J. Anat.* **121**, 285–304.

DORAI RAJ, B. S. & COHEN, M. J. (1962). Efferent control of a proprioceptive system in the crab leg. *Am. Zool.* **2**, 550.

DORAI RAJ, B. S. & COHEN, M. J. (1964). Structural and functional correlations in crab muscle fibers. *Naturwissenschaften* **9**, 224–5.

ECKERT, B. (1959). Uber das Zusammenwirken des erregenden und des hemmenden Neurons des M. abductor der Krebsschere beim Ablauf von Reflexen des myotätischen Typus. *Z. vergl. Physiol.* **41**, 500–26.

ELDRED, E., GRANIT, R. & MERTON, P. A. (1953). Supraspinal control of the muscle spindles and its significance. *J. Physiol.* **122**, 498–523.

FIELDS, H. L. (1966). Proprioceptive control of posture in the crayfish abdomen. *J. exp. Biol.* **44**, 455–68.

FIELDS, H. L., EVOY, W. H. & KENNEDY, D. (1967). Reflex role played by efferent control of an invertebrate stretch receptor. *J. Neurophysiol.* **30**, 859–74.

GRANIT, R., HOLMGREN, B. & MERTON, P. (1955). The two routes for excitation of muscle and their subservience to the cerebellum. *J. Physiol.* **130**, 213–24.

HWANG, J. C. C. (1961). The function of a second sensory cell group in the accessory flexor proprioceptive system of crab limbs. *Am. Zool.* **1**, 453.

LEKSELL, L. (1945). The action potential and excitatory effects of the small ventral root fibers to skeletal muscle. *Acta physiol. Scand.* **10**, Suppl. 31, 1–84.

PRINGLE, J. W. S. (1961). Proprioception in Arthropods. In *The Cell and the Organism* (Ed. J. A. Ramsay and V. B. Wigglesworth), Cambridge: University Press.

SMITH, R. I. (1947). The action of electrical stimulation and of certain drugs on cardiac nerves of the crab, *Cancer irroratus*. *Biol. Bull. mar. biol. Lab., Woods Hole* **93**, 72–88.

WHITEAR, M. (1962). The fine structure of crustacean proprioceptors. 1. The chordotonal organs in the legs of the shore crab, *Carcinus maenas*. *Phil. Trans. R. Soc.* B. **245**, 291–325.

WIERSMA, C. A. G. & BOETTIGER, E. G. (1959). Unidirectional movement fibres from a proprioceptive organ of a crab, *Carcinus maenas*. *J. exp. Biol.* **36** (1), 102–112.

WOLBARSHT, M. L., MACNICHOL, E. F. & WAGNER, H. G. (1960). Glass insulated microelectrodes. *Science, N.Y.* **132**, 1309–10.