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The Morphology and Ultrastructure of Tension Receptors in the Walking Legs of the Crab, *Carcinus maenas* *

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Summary. The tension receptor system of the crab merus consists of two size classes of receptor cell body distributed along one face of the flexor muscle apodeme. The receptors show the general arthropod mechanoreceptor structure of cell body, connecting cilium, and sheathed sensory processes, but there are several differences. Many processes show convolutions, and the distal portion of the sensory process is embedded in the apodeme cuticle. The terminations of the sensory processes lack the usual structural specialisations for mechanotransduction. Tension transduction appears to occur by flexion of the cuticle-embedded sensory process.

Key words: Neuroanatomy – Mechanoreceptor – Tension receptor – Cobalt sulphide – Sensory transduction.

The arthropods possess a variety of mechanoreceptors that are associated with the cuticle. Perhaps the best understood of these is the cockroach companiform sensilla, which provides information on external stress on the cuticle of the leg (Moran et al. 1971, 1976). Internal stress receptors, for example, leg apodeme tension receptors, have been described in crustaceans and xiphosurans, but only the gross morphology has been presented. Macmillan and Dando (1972) first described tension receptors on the apodemes of the flexor and extensor muscles in the crab *Cancer magister*. In whole-muscle preparations stained with methylene blue they found unbranched tension receptor dendrites innervating the tissues surrounding

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Eagles (1978) described the physiology of the limb tension receptors of the chelicerate *Limulus*, but included no information on the morphology of the receptors. Eagles and Hartman (1975) examined the tension receptor system of the tailspine musculature of *Limulus* in vivo and found two size classes of cell body involved in the innervation, but histological and ultrastructural details were not reported.

The present study describes the morphology and ultrastructure of the tension receptor system in the flexor muscle of *Carcinus maenas* (Linnaeus 1758) and investigates the relationship of these receptors to the muscle fibres and the apodeme. Possible modes of sensory transduction in this system are also discussed.

Materials and Methods

General

Specimens of *Carcinus maenas* (46–86 mm carapace width) were collected intertidally from Flinders and subtidally from Port Franklin, Victoria, and maintained in a filtered recirculating sea water system. Only healthy individuals with undamaged legs were used for experimentation. Walking legs were removed by induced autotomy. The cuticle on the anterior face of the merus (see Fig. 1 for anatomical nomenclature) was removed with a dental drill. The extensor muscle and apodeme were removed to gain access to the flexor apodeme and muscle. Dissections were bathed with crab saline at 7–10° C. The preparation of the saline followed the method of Smith and Ratcliffe (1978), however 0.05 M Hepes buffer (Sigma) was used instead of Tris buffer, and the saline was adjusted to pH 7.4–7.5. Methylene-blue staining of the gross innervation followed methods one and two of Wales et al. (1970).

Light Microscopy

The muscle and apodeme were removed and fixed in alcoholic Bouin's (Pantin 1964) containing 32 g/litre of NaCl. For ease of sectioning, some tissues were then decalcified for one to several days with saturated EGTA (Sigma) in 0.2 m phosphate buffer (pH = 7.4) or in a mixture of 5% formic acid and 5% formaldehyde (Humason 1972). The tissue was sectioned at 7–15 µm and stained by the silver impregnation method of Blest (1976).

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Thin sections were stained with methanolic uranyl acetate and lead citrate, and examined in a JEOL 100B electron microscope. Semi-thin sections (0.5 µm) were stained with the methylene-blue/basic fuchsin azure II (MBA) stain of Humphrey and Pitman (1974) or with a toluidine blue/basic fuchsin stain (Paragon Co. New York).

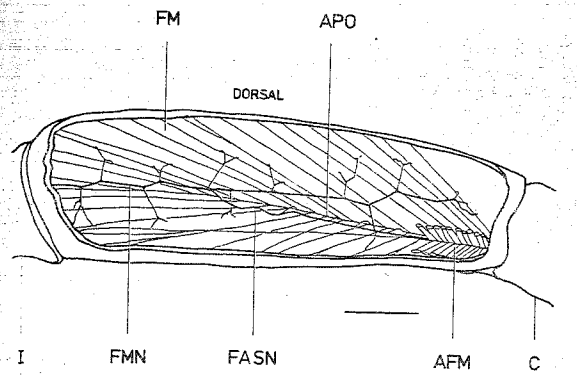

Fig. 1. Anterior view of merus flexor muscle and apodeme in left second walking leg of *C. maenas*. Extensor muscle, main leg nerve, and blood vessel have been removed to expose flexor muscle and associated nerves. Not all fine motor nerve branches have been drawn. Dorsal side of apodeme face designated the *axial* face; ventral side, the *peripheral* face. *AFM* Accessory Flexor Muscle; *APO* Apodeme; *C* Carpus; *FASN* Flexor Apodeme Sensory Nerve; *FM* Flexor Muscle; *FMN* Flexor Motor Nerve; *I* Ischium. Scale bar = 2.5 mm

Cobalt Backfilling

The cobalt backfilling method of Pitman et al. (1973) was followed but with several modifications. Cobalt acetate was found to be the most successful cobalt compound and was used at a concentration of 0.06 molar. The apodeme and muscle were removed, pinned out on wax in a small perspex dish, and barely covered with crab saline. A "mat" of a vaseline-paraffin oil mix was formed above the apodeme edge and the nerve lifted through. The nerve-stump was then freshly cut benath a drop of the cobalt solution. The preparation was refrigerated at 7° C for 8 to 36 h and the cobalt precipitated with ammonium sulphide. Following precipitation, the cobalt sulphide was progressively intensified with silver (Bacon and Altman 1977) and cleared in cedar wood oil. Control preparations (crab saline substituted for cobalt acetate) produced no precipitation or staining.

This procedure revealed fine dendritic endings only visible under the compound microscope, which provided excellent resolution but severely restricted the depth of field (dendritic endings typically traversed an 80 µm depth in the whole mount preparations). Hence, drawings of the stained dendrites were made by progressively focussing on the dendrite through its whole depth using the microscope stage calibrations to give an accurate spatial representation (Fig. 4).

Results

In *Carcinus*, the flexor muscle of the walking leg occupies the posterior half of the merus, and originates on the posterior, postero-dorsal, and postero-ventral walls of the merus. The muscle inserts onto the flexor apodeme, which originates at the ventral and proximal end of the carpus. The joint is hinged antero-posteriorly. Associated with the flexor apodeme insertion is a much smaller muscle, the accessory flexor. This muscle inserts, via its own apodeme, onto the anterior edge of the flexor apodeme at the merus-carpus (MC) joint (Fig. 1).

Gross Innervation

The main leg nerve (MLN) and blood vessel run together approximately axially through the merus. The MLN contains the Flexor Apodeme Sensory Nerve

(FASN) which runs together with the Flexor Motor Nerve (FMN) bundle in the proximal half of the merus. The FASN separates from the FMN bundle approximately midway along the length of the merus. At this point the FASN bundle consists of approximately eight 14–20 µm diameter fibres, ten 9–12 µm fibres, and numerous small (< 5 µm) fibres (Fig. 2). The FASN descends via an "S" bend to the inner (anterior) edge of the flexor apodeme. The "S" bend prevents strain on the FASN during displacements of the flexor apodeme that occur with carpus extension and flexion. The FASN then runs along the apodeme edge towards the M-C joint area.

At several points along the FASN (usually four) single axons branch off and run across the axial (dorsal) face of the apodeme towards the posterior edge, and some branches curve around the edges of muscle bundles. Each of these branches runs to a large (typically 70 µm), bipolar cell body close to the axial apodeme face (Fig. 3). Nearer to the M-C joint, the remainder of the FASN gives rise to numerous smaller branches that contain numbers of fine fibres. These fibres innervate approximately the distal third of the flexor apodeme, and possess small (typically < 30 µm) bipolar cell bodies (Fig. 3). The large, more proximal cell bodies are designated Proximal Sensory Cells (PSC) and the smaller, the Distal Sensory Cells (DSC), following the terminology of Macmillan and Dando (1972).

Most of the innervation from the FASN is on the axial face of the apodeme; however, rarely a PSC branch occurs on the peripheral (ventral) face. Similarly, some fine distal fibres of the FASN have been found on the peripheral face near the M-C joint, however the majority run along and across the axial face of the apodeme adjacent to the insertion of the accessory flexor apodeme into the M-C joint area.

b) Dendrites of the PSC's and DSC's

Only the gross features of the innervation pattern could be observed in the methylene blue stained preparations, because many of the fine DSC's, and particularly the dendritic terminations of both these and PSC's stain poorly and irregularly with methylene blue. The tight packing of some of the muscle bundles in particular restricts the penetration of the methylene blue into the fine endings in the hypodermis near the insertions of these muscles.

Cobalt backfilling of the FASN, followed by silver intensification of the precipitated cobalt sulphide revealed fine dendritic endings (less than 0.5 µm in diameter), although this was dependent on both the success of the cobalt impregnation and, again, the positioning of the surrounding muscle insertions. Up to 150 cobalt filled dendritic endings have been observed on the apodeme in whole mount preparations. Since not all endings would be expected to be filled in a given preparation, this figure is likely to be conservative.

Because the nerve fibres ran both through and around the edges of muscle insertions, these muscles were progressively removed during the intensification procedure to allow the nerves, cell bodies, and endings to be seen. Although this method provided excellent detail of the dendrites themselves, it disrupted or destroyed the relationships of the dendrites to their cell bodies, to the individual muscles, and to the FASN branches. Figure 4 shows an example of two dendrites of tension receptor cells.

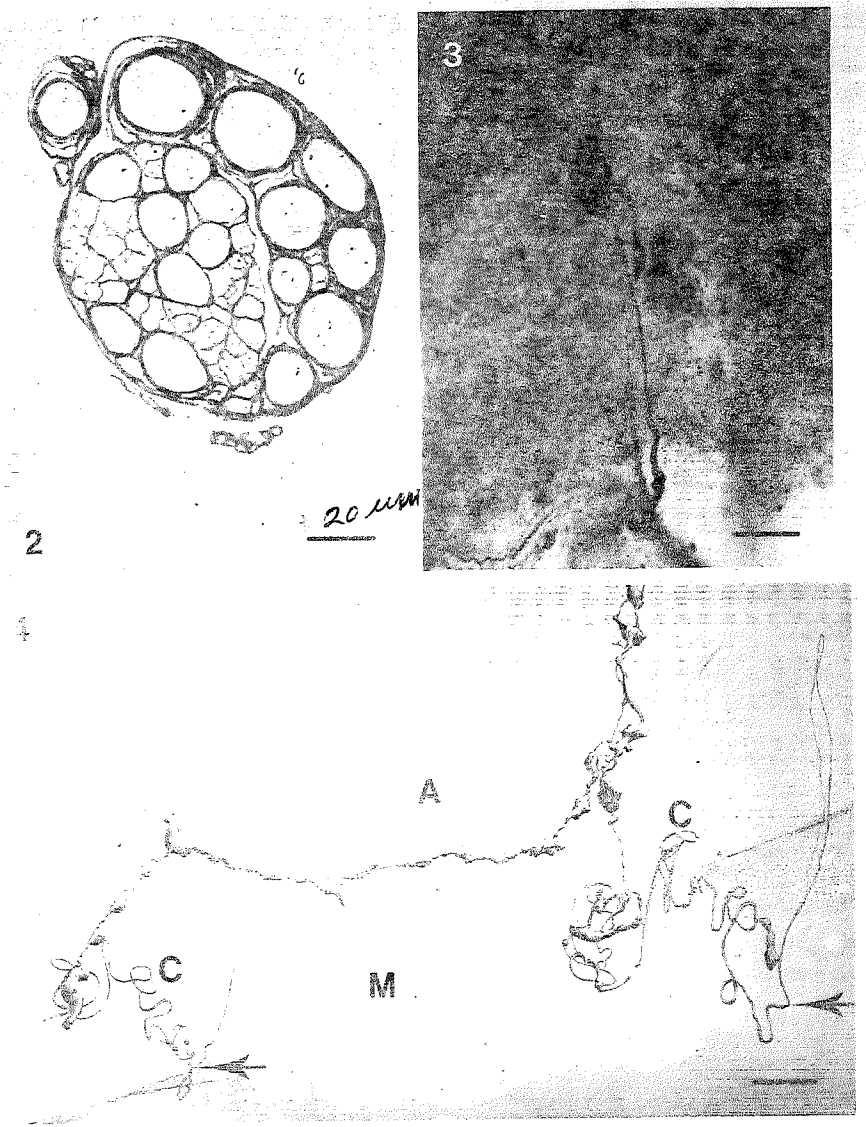

Fig. 2. Semi-thin cross section of FASN. Note three general size classes of fibre. Toluidine blue stain. Scale bar = 20 µm

Fig. 3. PSC and adjacent smaller DSC in hypodermal layer of merus flexor apodeme. Both cells oriented with their axons uppermost. Cobalt fill. Scale bar = 50 µm

Fig. 4. Drawing of two tension receptor dendrites on flexor apodeme (axial face view). Note complex convolution (*C*) of dendrites adjacent to their point of entry (*arrow*) into apodeme. Distal to arrow dendrite runs smoothly, with one change in direction, until its termination. Sections of this distal portion (*SP*) and of convolutions are seen in Figs. 6 and 7, respectively. Cobalt-filled, silver intensified preparation. *A* apodeme; *M* edge of muscle fibre insertion. Scale bar = 10 µm

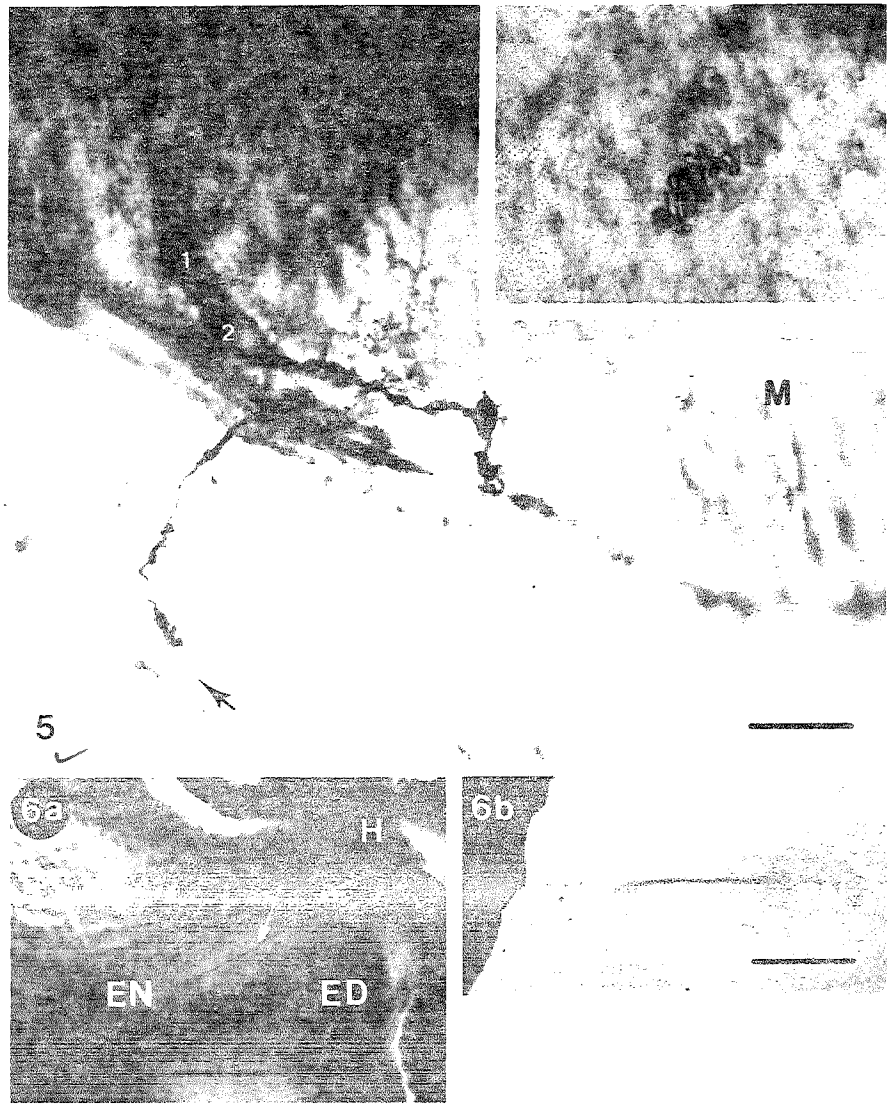

Fig. 5. Photomicrograph of wholemount of tension receptors on face of apodeme of *Carcinus* flexor muscle. One DSC body (*1*) partially obscures the other (*2*). *Inset:* More distal portion of dendrite 2 (*arrow*) at same magnification, showing convolutions. Cobalt filled and silver intensified preparation. Scale bar = 20 µm

Fig. 6 a and b. Apodeme Confined Sensory Process (ACSP) in *C. maenas*. **a** Cross-section showing ACSP (*arrow*) within apodeme. Note papilla-like protrusion of apodeme face at this point. ACSP surrounded by a lightly stained envelope. Blest silver stain. **b** Semi-thin cross-section of flexor apodeme containing ACSP and surrounding envelope, as seen in **a**. MBA stain. In both cases, left end of ACSP oriented towards epicuticular suture line. *H* hypodermis; *ED* endocuticle; *EN* envelope. Scale bars = 20 µm

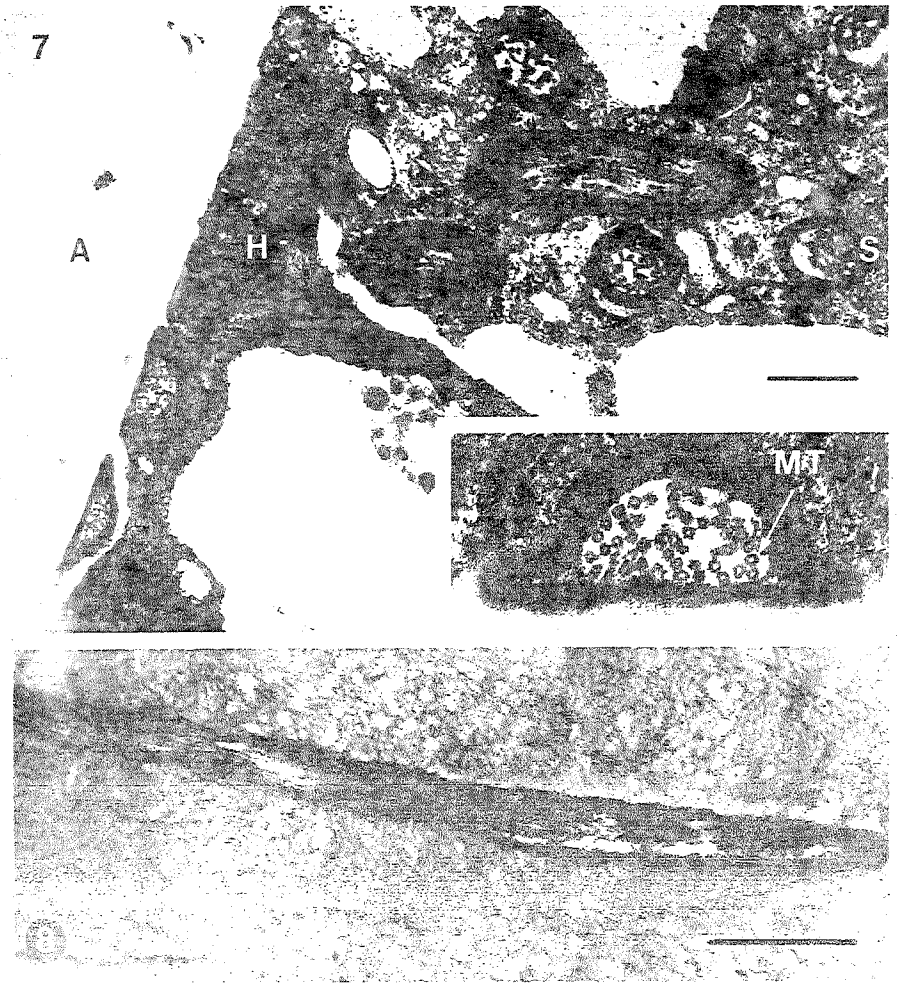

Fig. 7. Cross section through the highly convoluted sensory process of single tension receptor unit in hypodermis of *Carcinus* flexor muscle apodeme. *Inset:* One portion of sensory process, situated on epicuticle-hypodermis junction. *A* apodeme cuticle; *H* hypodermis; *MT* microtubules; *S* electron-opaque sheath. Scale bar = 0.5 µm. *Inset* = 0.06 µm

Fig. 8. Slightly oblique longitudinal section of tension-receptor sensory process near its termination in apodeme. Note absence of space between process and surrounding cuticle. Scale bar = 1 µm

The receptor dendrites run in the hypodermis and connective tissue between muscle bundles, and in some cases run through the edges of the muscle insertions on the apodeme. The dendrites then descend to the myo-apodeme junction and in most cases undergo complex coiling for a depth of 5–10 µm in the hypodermal tissue before entering the apodeme (Fig. 5). The entry point of the dendrite into the apodeme is usually associated with a papilla-like protrusion of the apodeme edge (Fig. 6a). The fibre then runs through the endocuticle towards the epicuticular layer

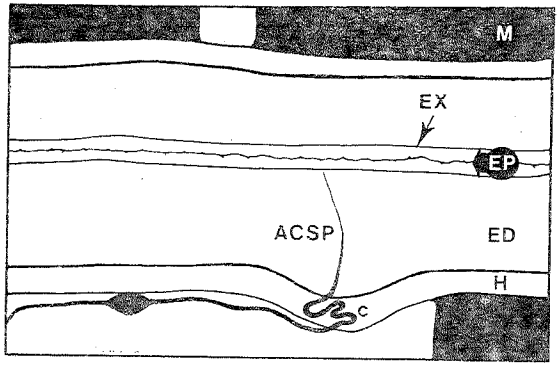

Fig. 9. Diagram of generalised tension-receptor cell body and sensory process (not to scale). Sensory process runs along hypodermal layer (*H*) and enters apodeme via convolution (*c*) within hypodermis. Process (*ACSP*) then runs through endocuticle (*ED*) and terminates near epicuticular suture line (*EP*) between thin exocuticle (*EX*) layers. M muscle fibre

(Fig. 6b) and terminates near the epicuticular suture line between the two invaginated faces of cuticle that form the apodeme. The portions of the tension receptor dendrites found within the apodemes are designated the Apodeme Confined Sensory Processes (ACSP's). The apodeme is not symmetrical in cross section. In the distal half of the apodeme, the endocuticle layer on the axial side of the suture line is wider than that on the opposite side.

Ultrastructure

Ultrastructural study of these dendrites shows they are the sensory processes of the tension receptors. The processes are bounded by an electron-opaque sheath and are filled with microtubules aligned with the long axis of the process (Fig. 7). Distal to the cell body each receptor possesses a single connecting cilium (a "9 + 0" structure), a feature of a wide range of arthropod receptors (McIver 1975). The connecting cilium is surrounded by an extracellular space, and develops distally into the sheathed sensory process. The processes run parallel to the apodeme in the hypodermal cell layer for varying distances before entering the apodeme and many of the sensory processes are highly convoluted here (Fig. 7). The portion of the sensory process lying within the apodeme (the ACSP) is completely embedded in the cuticle, with no extracellular space between the electron opaque sheath and the cuticle (Fig. 8). The diameter of the sensory process decreases as the process runs towards the suture line of the apodeme. The distal terminations of the processes contain small amounts of electron dense material, but discrete areas of microtubules together with electron-dense material (i.e., the tubular bodies of other arthropod mechanoreceptors) have not been observed (McIver 1975; Moran et al. 1976; French and Sanders 1979). Some receptors are positioned with their cell bodies immediately adjacent to the process entry point, so that the whole of the sensory process is embedded in the cuticle; none of the process runs along the hypodermal layer. Figure 9 shows a generalised diagram of a receptor.

A line of disrupted cuticle extends from the termination of the sensory process into the junction of the two opposing epicuticular surfaces of the apodeme. This

extension contains no microtubules or cellular components and is probably the degenerated path of the sensory process that connects the new ACSP to the old ACSP during moulting.

Discussion

It is apparent that the fine structure of these tension receptors is similar to that of the cockroach campaniform sensilla (Moran et al. 1971) in most respects, but with two important differenees. First, there is no discrete structure at the distal end of the sensory process. In other arthropod mechanoreceptors, the distal end of the sensory process is important in the transduction process. In the cockroach campaniform sensilla for example, the convex two-layered cuticular cap is considered to be the site of sensory transduction, and is arranged to act as a mechanical amplifier (Moran et al. 1976). The second difference is that after entering the apodeme, the sensory process is in direct contact with the cuticle at all times. This arrangement means that any forces within the apodeme cuticle bear directly on the sensory process. In contrast, the sensory process of the campaniform sensilla runs within a separate canal in the cuticle until reaching the cap at the distal end of the process (Moran et al. 1971).

These differences in structure imply that transduction occurs differently in these receptors. The absence of a discrete structure at the distal tip of the dendrite suggests that the mechanical changes that occur with changes in tension in the leg stimulate the receptor elsewhere. Physiological investigations of the system have shown that bending of the apodeme is the mechanical change that produces afferent activity in the apodeme sensory nerve (Parsons, in preparation). The sufficient stimulus for these receptors thus appears to be the bending of the apodeme which is caused by tension in the attached muscle. It is therefore likely that transduction by cell membrane deformation is occurring along the whole length of the cuticle-embedded sensory process (ACSP). A tubular body is not involved in cell membrane deformation here, but given the similarities in receptor structure described earlier, subsequent events in the transduction process (French and Sanders 1979) are likely to be the same as those postulated for receptors that do possess tubular bodies. The hypothesis that transduction occurs as a result of sensory process flexion has also been suggested for an external mechanoreceptive sensilla on the tsetse fly labella (Rice et al. 1973).

A striking feature of this system is the convolution of many of the receptor dendrites just prior to their entry into the apodeme cuticle. Moran et al. (1976) have described coiled sensory processes in the campaniform sensilla, but these are present apparently only during the molting cycle. French and Sanders (1979) note the presence of a convoluted dendrite portion just proximal to the fibrous dome in the cockroach hair plate sensilla, but offer no suggestions as to the function. Although the functional significance of this convolution has not been shown here, it is likely that it protects the dendrites from stretch and strain during the considerable muscle and apodeme movements resulting from both active flexor contraction and passive movement. The presence of these convolutions argues

against the involvement of active microtubule sliding in the transduction process here (French and Sanders 1979).

The apodeme, derived from the fusion of two opposed layers of invaginated cuticle, can be regarded as a beam that bends as a result of tension in the attached muscle. Simple beam theory states that a flexed beam will be compressed on one face and be under tension on the opposite face. At some plane within the beam (the neutral axis) neither compression nor tension is experienced (Wainwright 1976). In homogeneous materials, this axis will lie on the centre line of the beam. The exact position of the neutral axis in the non-homogeneous apodeme depends on the relative strengths of the constitutent cuticle layers. Since the layers in the apodeme show only slight asymmetry about the suture line, the neutral axis will lie near the centre of the apodeme. Apodeme distortion as a result of muscle contraction will be greatest at the outer face of the apodeme. This gradation in the degree of distortion may be the reason for the gradation of ACSP diameter: a matching of these two factors would maximise receptor sensitivity.

Campaniform-like organs have been found in the external cuticle of *Carcinus* (Shelton and Laverack 1968), but these organs are doubly innervated, which suggests closer affinities with the internal chordotonal organs than with the singly innervated campaniform receptors. No ultrastructural details have been presented for the campaniform-like organs in the crab so that no direct comparisons can be made at this stage.

Physiological and ultrastructural investigations are in progress to determine the functional differences between the PSC and DSC classes, and to provide more detailed information on the transduction process and on the importance of these receptors in walking leg proprioception.

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Thin sections were stained with methanolic uranyl acetate and lead citrate, and examined in a JEOL 100B electron microscope. Semi-thin sections (0.5 µm) were stained with the methylene-blue/basic fuchsin azure II (MBA) stain of Humphrey and Pitman (1974) or with a toluidine blue/basic fuchsin stain (Paragon Co. New York).

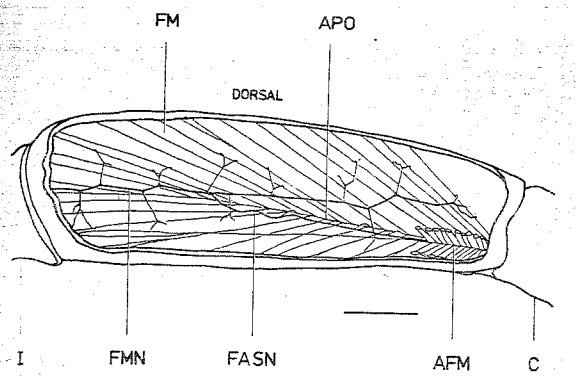


Fig. 1. Anterior view of merus flexor muscle and apodeme in left second walking leg of *C. maenas*. Extensor muscle, main leg nerve, and blood vessel have been removed to expose flexor muscle and associated nerves. Not all fine motor nerve branches have been drawn. Dorsal side of apodeme face designated the *axial* face; ventral side, the *peripheral* face. *AFM* Accessory Flexor Muscle; *APO* Apodeme; *C* Carpus; *FASN* Flexor Apodeme Sensory Nerve; *FM* Flexor Muscle; *FMN* Flexor Motor Nerve; *I* Ischium. Scale bar = 2.5 mm

Cobalt Backfilling

The cobalt backfilling method of Pitman et al. (1973) was followed but with several modifications. Cobalt acetate was found to be the most successful cobalt compound and was used at a concentration of 0.06 molar. The apodeme and muscle were removed, pinned out on wax in a small perspex dish, and barely covered with crab saline. A "mat" of a vaseline-paraffin oil mix was formed above the apodeme edge and the nerve lifted through. The nerve-stump was then freshly cut benath a drop of the cobalt solution. The preparation was refrigerated at 7° C for 8 to 36 h and the cobalt precipitated with ammonium sulphide. Following precipitation, the cobalt sulphide was progressively intensified with silver (Bacon and Altman 1977) and cleared in cedar wood oil. Control preparations (crab saline substituted for cobalt acetate) produced no precipitation or staining.

This procedure revealed fine dendritic endings only visible under the compound microscope, which provided excellent resolution but severely restricted the depth of field (dendritic endings typically traversed an 80 µm depth in the whole mount preparations). Hence, drawings of the stained dendrites were made by progressively focussing on the dendrite through its whole depth using the microscope stage calibrations to give an accurate spatial representation (Fig. 4).

Results

In *Carcinus*, the flexor muscle of the walking leg occupies the posterior half of the merus, and originates on the posterior, postero-dorsal, and postero-ventral walls of the merus. The muscle inserts onto the flexor apodeme, which originates at the ventral and proximal end of the carpus. The joint is hinged antero-posteriorly. Associated with the flexor apodeme insertion is a much smaller muscle, the accessory flexor. This muscle inserts, via its own apodeme, onto the anterior edge of the flexor apodeme at the merus-carpus (MC) joint (Fig. 1).

Gross Innervation

The main leg nerve (MLN) and blood vessel run together approximately axially through the merus. The MLN contains the Flexor Apodeme Sensory Nerve

(FASN) which runs together with the Flexor Motor Nerve (FMN) bundle in the proximal half of the merus. The FASN separates from the FMN bundle approximately midway along the length of the merus. At this point the FASN bundle consists of approximately eight 14–20 µm diameter fibres, ten 9–12 µm fibres, and numerous small (< 5 µm) fibres (Fig. 2). The FASN descends via an "S" bend to the inner (anterior) edge of the flexor apodeme. The "S" bend prevents strain on the FASN during displacements of the flexor apodeme that occur with carpus extension and flexion. The FASN then runs along the apodeme edge towards the M-C joint area.

At several points along the FASN (usually four) single axons branch off and run across the axial (dorsal) face of the apodeme towards the posterior edge, and some branches curve around the edges of muscle bundles. Each of these branches runs to a large (typically 70 µm), bipolar cell body close to the axial apodeme face (Fig. 3). Nearer to the M-C joint, the remainder of the FASN gives rise to numerous smaller branches that contain numbers of fine fibres. These fibres innervate approximately the distal third of the flexor apodeme, and possess small (typically < 30 µm) bipolar cell bodies (Fig. 3). The large, more proximal cell bodies are designated Proximal Sensory Cells (PSC) and the smaller, the Distal Sensory Cells (DSC), following the terminology of Macmillan and Dando (1972).

Most of the innervation from the FASN is on the axial face of the apodeme; however, rarely a PSC branch occurs on the peripheral (ventral) face. Similarly, some fine distal fibres of the FASN have been found on the peripheral face near the M-C joint, however the majority run along and across the axial face of the apodeme adjacent to the insertion of the accessory flexor apodeme into the M-C joint area.

b) Dendrites of the PSC's and DSC's

Only the gross features of the innervation pattern could be observed in the methylene blue stained preparations, because many of the fine DSC's, and particularly the dendritic terminations of both these and PSC's stain poorly and irregularly with methylene blue. The tight packing of some of the muscle bundles in particular restricts the penetration of the methylene blue into the fine endings in the hypodermis near the insertions of these muscles.

Cobalt backfilling of the FASN, followed by silver intensification of the precipitated cobalt sulphide revealed fine dendritic endings (less than 0.5 µm in diameter), although this was dependent on both the success of the cobalt impregnation and, again, the positioning of the surrounding muscle insertions. Up to 150 cobalt filled dendritic endings have been observed on the apodeme in whole mount preparations. Since not all endings would be expected to be filled in a given preparation, this figure is likely to be conservative.

Because the nerve fibres ran both through and around the edges of muscle insertions, these muscles were progressively removed during the intensification procedure to allow the nerves, cell bodies, and endings to be seen. Although this method provided excellent detail of the dendrites themselves, it disrupted or destroyed the relationships of the dendrites to their cell bodies, to the individual muscles, and to the FASN branches. Figure 4 shows an example of two dendrites of tension receptor cells.

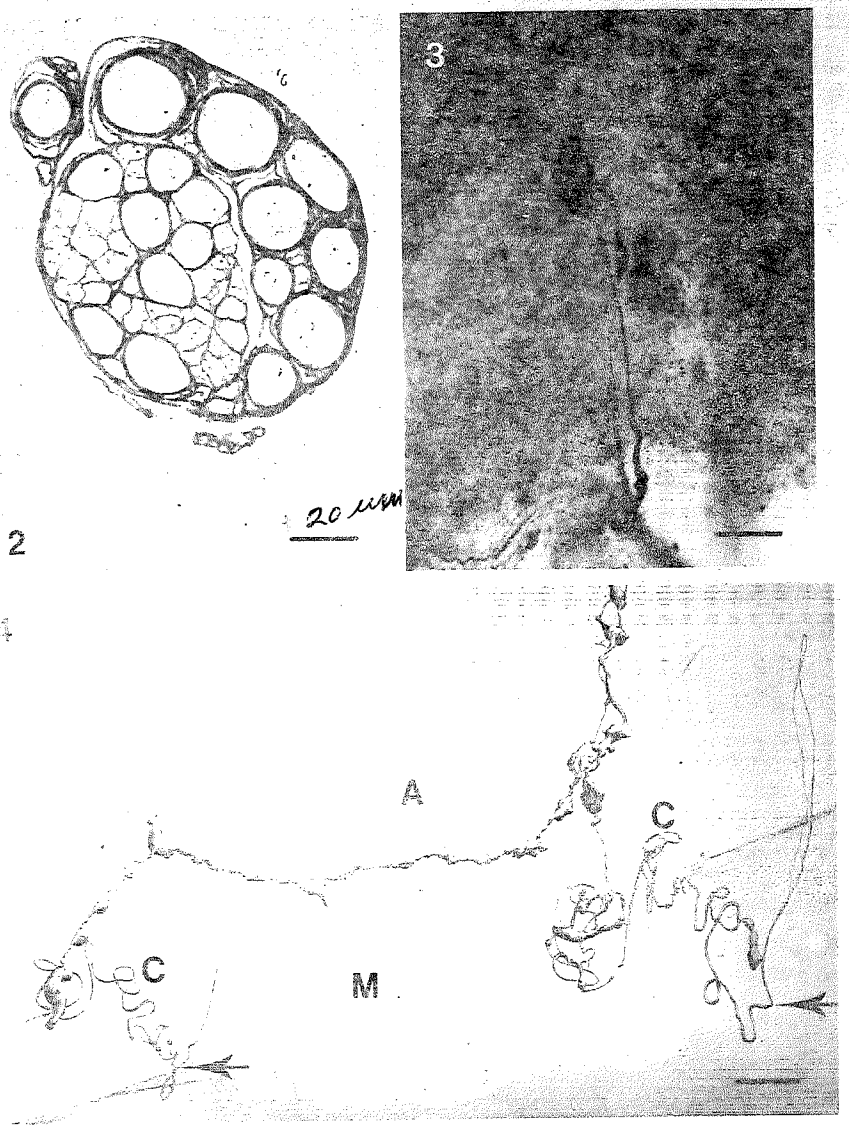


Fig. 2. Semi-thin cross section of FASN. Note three general size classes of fibre. Toluidine blue stain. Scale bar = 20 µm

Fig. 3. PSC and adjacent smaller DSC in hypodermal layer of merus flexor apodeme. Both cells oriented with their axons uppermost. Cobalt fill. Scale bar = 50 µm

Fig. 4. Drawing of two tension receptor dendrites on flexor apodeme (axial face view). Note complex convolution (*C*) of dendrites adjacent to their point of entry (*arrow*) into apodeme. Distal to arrow fibre runs smoothly, with one change in direction, until its termination. Sections of this distal portion (SP) and of convolutions are seen in Figs. 6 and 7, respectively. Cobalt-filled, silver intensified preparation. *A* apodeme; *M* edge of muscle fibre insertion. Scale bar = 10 µm

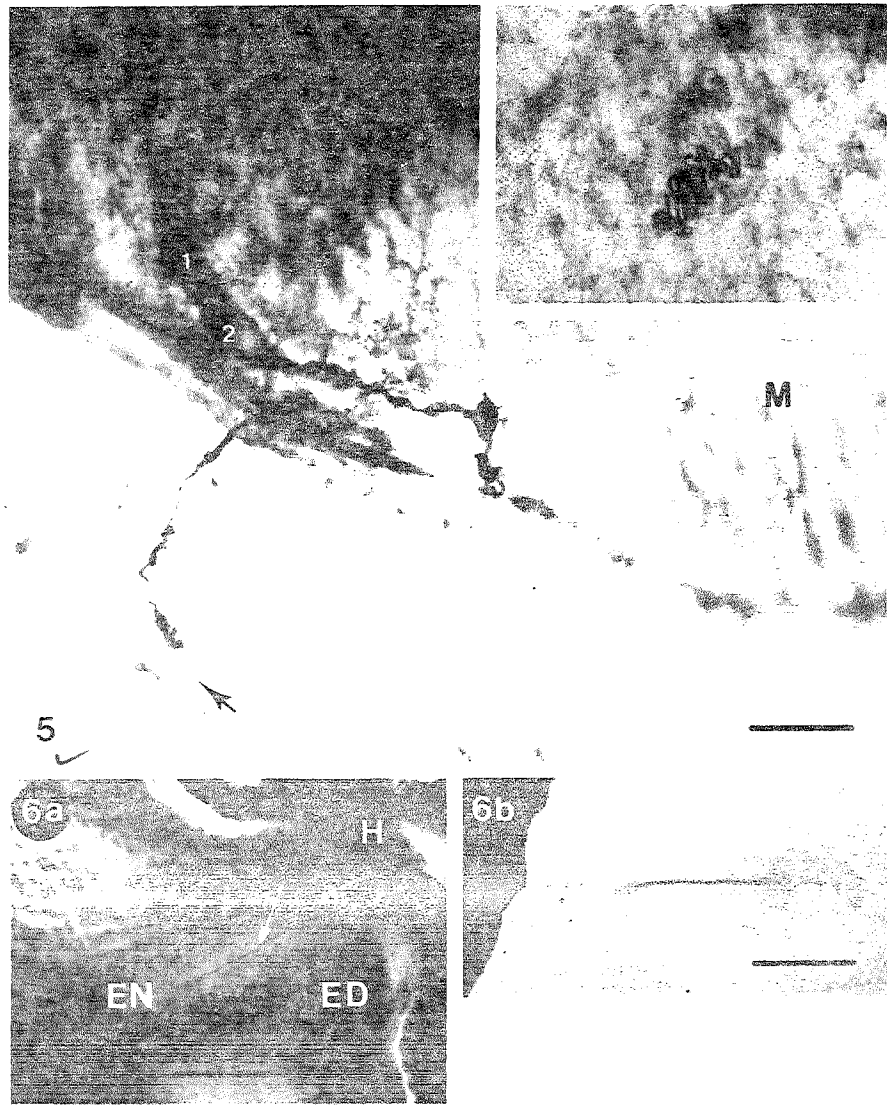
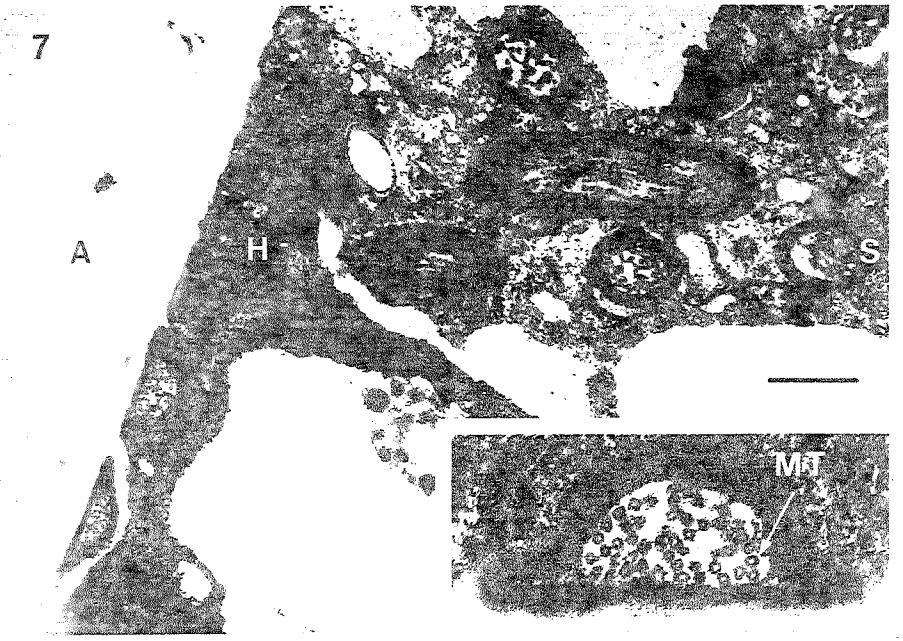


Fig. 5. Photomicrograph of wholemount of tension receptors on face of apodeme of *Carcinus* flexor muscle. One DSC body (*1*) partially obscures the other (*2*). *Inset:* More distal portion of dendrite 2 (arrow) at same magnification, showing convolutions. Cobalt filled and silver intensified preparation. Scale bar = 20 µm

Fig. 6 a and b. Apodeme Confined Sensory Process (ACSP) in *C. maenas*. **a** Cross-section showing ACSP (arrow) within apodeme. Note papilla-like protrusion of apodeme face at this point. ACSP surrounded by a lighter stained envelope. Blest silver stain. **b** Semi-thin cross-section of flexor apodeme containing ACSP and surrounding envelope, as seen in **a**. MBA stain. In both cases, left end of ACSP oriented towards epicuticular suture line. *H* hypodermis; *ED* endocuticle; *EN* envelope. Scale bars = 20 µm



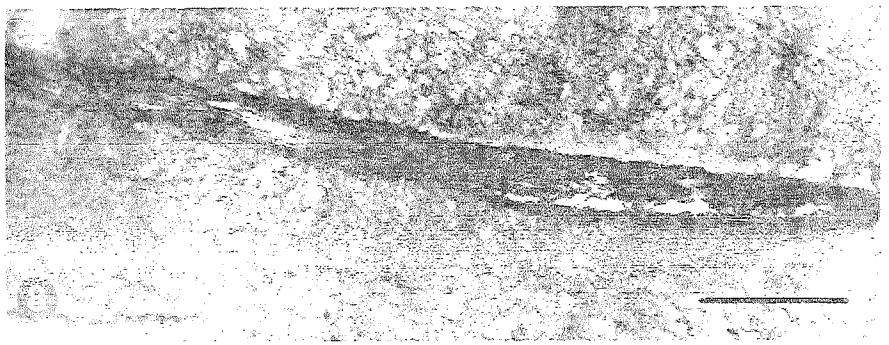


Fig. 7. Cross section through the highly convoluted sensory process of single tension receptor unit in hypodermis of *Carcinus* flexor muscle apodeme. *Inset:* One portion of sensory process, situated on apodeme-hypodermis junction. *A* apodeme cuticle; *H* hypodermis; *MT* microtubules; *S* electron-opaque sheath. Scale bar = 0.5 µm. *Inset* = 0.06 µm

Fig. 8. Slightly oblique longitudinal section of tension-receptor sensory process near its termination in apodeme. Note absence of space between process and surrounding cuticle. Scale bar = 1 µm

The receptor dendrites run in the hypodermis and connective tissue between muscle bundles, and in some cases run through the edges of the muscle insertions on the apodeme. The dendrites then descend to the myo-apodeme junction and in most cases undergo complex coiling for a depth of 5–10 µm in the hypodermal tissue before entering the apodeme (Fig. 5). The entry point of the dendrite into the apodeme is usually associated with a papilla-like protrusion of the apodeme edge (Fig. 6a). The fibre then runs through the endocuticle towards the epicuticular layer

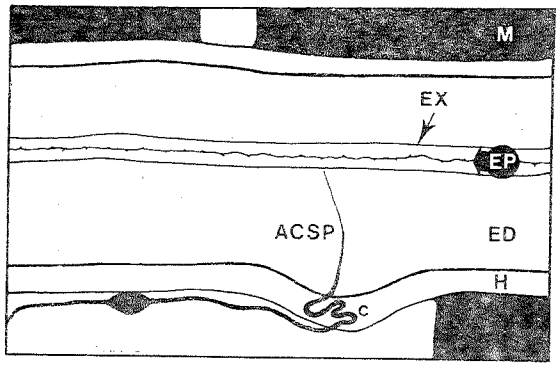


Fig. 9. Diagram of generalised tension-receptor cell body and sensory process (not to scale). Sensory process runs along hypodermal layer (*H*) and enters apodeme via convolution (*c*) within hypodermis. Process (*ACSP*) then runs through endocuticle (*ED*) and terminates near epicuticular suture line (*EP*) between thin exocuticle (*EX*) layers. M muscle fibre

(Fig. 6b) and terminates near the epicuticular suture line between the two invaginated faces of cuticle that form the apodeme. The portions of the tension receptor dendrites found within the apodemes are designated the Apodeme Confined Sensory Processes (ACSP's). The apodeme is not symmetrical in cross section. In the distal half of the apodeme, the endocuticle layer on the axial side of the suture line is wider than that on the opposite side.

Ultrastructure

Ultrastructural study of these dendrites shows they are the sensory processes of the tension receptors. The processes are bounded by an electron-opaque sheath and are filled with microtubules aligned with the long axis of the process (Fig. 7). Distal to the cell body each receptor possesses a single connecting cilium ("9 + 0" structure), a feature of a wide range of arthropod receptors (McIver 1975). The connecting cilium is surrounded by an extracellular space, and develops distally into the sheathed sensory process. The processes run parallel to the apodeme in the hypodermal cell layer for varying distances before entering the apodeme and many of the sensory processes are highly convoluted here (Fig. 7). The portion of the sensory process lying within the apodeme (the ACSP) is completely embedded in the cuticle, with no extracellular space between the electron opaque sheath and the cuticle (Fig. 8). The diameter of the sensory process decreases as the process runs towards the suture line of the apodeme. The distal terminations of the processes contain small amounts of electron dense material, but discrete areas of microtubules together with electron-dense material (i.e., the tubular bodies of other arthropod mechanoreceptors) have not been observed (McIver 1975; Moran et al. 1976; French and Sanders 1979). Some receptors are positioned with their cell bodies immediately adjacent to the process entry point, so that the whole of the sensory process is embedded in the cuticle; none of the process runs along the hypodermal layer. Figure 9 shows a generalised diagram of a receptor.

A line of disrupted cuticle extends from the termination of the sensory process into the junction of the two opposing epicuticular surfaces of the apodeme. This

extension contains no microtubules or cellular components and is probably the degenerated path of the sensory process that connects the new ACSP to the old ACSP during moulting.

Discussion

It is apparent that the fine structure of these tension receptors is similar to that of the cockroach campaniform sensilla (Moran et al. 1971) in most respects, but with two important differenees. First, there is no discrete structure at the distal end of the sensory process. In other arthropod mechanoreceptors, the distal end of the sensory process is important in the transduction process. In the cockroach campaniform sensilla for example, the convex two-layered cuticular cap is considered to be the site of sensory transduction, and is arranged to act as a mechanical amplifier (Moran et al. 1976). The second difference is that after entering the apodeme, the sensory process is in direct contact with the cuticle at all times. This arrangement means that any forces within the apodeme cuticle bear directly on the sensory process. In contrast, the sensory process of the campaniform sensilla runs within a separate canal in the cuticle until reaching the cap at the distal end of the process (Moran et al. 1971).

These differences in structure imply that transduction occurs differently in these receptors. The absence of a discrete structure at the distal tip of the dendrite suggests that the mechanical changes that occur with changes in tension in the leg stimulate the receptor elsewhere. Physiological investigations of the system have shown that bending of the apodeme is the mechanical change that produces afferent activity in the apodeme sensory nerve (Parsons, in preparation). The sufficient stimulus for these receptors thus appears to be the bending of the apodeme which is caused by tension in the attached muscle. It is therefore likely that transduction by cell membrane deformation is occurring along the whole length of the cuticle-embedded sensory process (ACSP). A tubular body is not involved in cell membrane deformation here, but given the similarities in receptor structure described earlier, subsequent events in the transduction process (French and Sanders 1979) are likely to be the same as those postulated for receptors that do possess tubular bodies. The hypothesis that transduction occurs as a result of sensory process flexion has also been suggested for an external mechanoreceptive sensilla on the tsetse fly labella (Rice et al. 1973).

A striking feature of this system is the convolution of many of the receptor dendrites just prior to their entry into the apodeme cuticle. Moran et al. (1976) have described coiled sensory processes in the campaniform sensilla, but these are present apparently only during the molting cycle. French and Sanders (1979) note the presence of a convoluted dendrite portion just proximal to the fibrous dome in the cockroach hair plate sensilla, but offer no suggestions as to the function. Although the functional significance of this convolution has not been shown here, it is likely that it protects the dendrites from stretch and strain during the considerable muscle and apodeme movements resulting from both active flexor contraction and passive movement. The presence of these convolutions argues

against the involvement of active microtubule sliding in the transduction process here (French and Sanders 1979).

The apodeme, derived from the fusion of two opposed layers of invaginated cuticle, can be regarded as a beam that bends as a result of tension in the attached muscle. Simple beam theory states that a flexed beam will be compressed on one face and be under tension on the opposite face. At some plane within the beam (the neutral axis) neither compression nor tension is experienced (Wainwright 1976). In homogeneous materials, this axis will lie on the centre line of the beam. The exact position of the neutral axis in the non-homogeneous apodeme depends on the relative strengths of the constitutent cuticle layers. Since the layers in the apodeme show only slight asymmetry about the suture line, the neutral axis will lie near the centre of the apodeme. Apodeme distortion as a result of muscle contraction will be greatest at the outer face of the apodeme. This gradation in the degree of distortion may be the reason for the gradation of ACSP diameter: a matching of these two factors would maximise receptor sensitivity.

Campaniform-like organs have been found in the external cuticle of *Carcinus* (Shelton and Laverack 1968), but these organs are doubly innervated, which suggests closer affinities with the internal chordotonal organs than with the singly innervated campaniform receptors. No ultrastructural details have been presented for the campaniform-like organs in the crab so that no direct comparisons can be made at this stage.

Physiological and ultrastructural investigations are in progress to determine the functional differences between the PSC and DSC classes, and to provide more detailed information on the transduction process and on the importance of these receptors in walking leg proprioception.

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