J. exp. Biol. **163**, 345–358 (1992)
Printed in Great Britain © *The Company of Biologists Limited 1992*

THE APODEME COMPLEX OF THE FEMORAL CHORDOTONAL ORGAN IN THE METATHORACIC LEG OF THE LOCUST *SCHISTOCERCA GREGARIA*

BY P. M. J. SHELTON[1], R. O. STEPHEN[2], J. J. A. SCOTT[2] AND A. R. TINDALL[1]

[1]*Department of Zoology, Adrian Building, University of Leicester, Leicester LE1 7RH, UK* and [2]*Department of Physiology, The Medical School, University of Leicester, Leicester LE1 7RH, UK*

Accepted 28 October 1991

Summary

The mechanical connections of the metathoracic femoral chordotonal organ (mtFCO) with its insertion at the femoro-tibial joint are described. The apodeme complex is shown to consist of a distal cuticular rod that is replaced proximally by dorsal and ventral ligaments. The dorsal ligament is a direct continuation of the distal rod but proximally it is replaced by ligamentous cells. The ventral ligament has no cuticular core and consists of ligamentous cells throughout its length. The ligaments are composed of bundles of connective tissue cells that are each enclosed in an extracellular matrix containing acid-fuchsin-staining fibrils. Internally the cells are packed with microtubules. During extension and flexion of the joint, the two ligaments move differentially. During passive extension of the tibia, the ventral ligament remains taut but the dorsal one buckles to form a slack loop. Direct observation of living preparations shows that the loop is first detectable during extension of the tibia at joint angles greater than about 51°. During flexion, the loop gradually tightens and disappears. It has completely disappeared at joint angles of less than about 36°. Changes in loop shape were demonstrable using preparations in which the tibia was moved sinusoidally ±10° about a mean femoro-tibial angle of 90° and photographs were taken using phase-locked illumination. Other details of the apodeme complex are described and the significance of the findings is discussed in relation to mtFCO function.

Introduction

In proprioceptors such as the metathoracic femoral chordotonal organ (mtFCO) of the locust, the response properties of the sensory neurones may be determined partly by the properties of the receptor cells themselves and partly by the mechanical properties of those elements that link the receptor to the joint whose position and movement they monitor (Usherwood *et al.* 1968; Zill, 1985; Matheson, 1990; Matheson and Field, 1990). In addition, the sense organ may be

Key words: chordotonal organ, locust, apodeme complex, *Schistocerca gregaria*.

mechanically linked to some or all of the muscles that bring about joint movements. In that case, the response properties of the sense organ may vary with the state of contraction in those muscles. With respect to the femoral chordotonal organ, the relative importance of each of these factors in determining chordotonal organ output is very poorly understood. Our knowledge of mtFCO biomechanics is virtually non-existent and it is our intention to close this gap. We have begun to investigate the relationship between the properties of the mtFCO sensory neurones and the mechanical movements of different parts of the organ and associated structures. Our initial investigations are directed at the structure and behaviour of the apodeme complex during passive flexion and extension. We show that the apodeme complex is a stucture of hitherto unsuspected complexity. Its organisation and behaviour are so unusual and of such obvious functional importance that a description of the major features is needed. Future studies will be directed towards a thorough analysis of the biomechanical properties of the various components described in this paper. Previous studies show that the body of the organ, located in the distal femur, is firmly anchored to its outer (anterior) wall (Usherwood *et al.* 1968; Zill, 1985). Distally, the apodeme inserts onto the base of the tibia adjacent to the insertion of the extensor tibiae muscle. In addition, there is also a distal ligamentous flexor strand attachment to the apodeme of the flexor tibiae muscle (Field and Burrows, 1982; Zill, 1985). This has been shown to be associated with a strand receptor (Bräunig, 1985). Previously, numerous other smaller connective tissue ligaments arising from the body of the mtFCO and the apodeme were noted (Usherwood *et al.* 1968). These were described as being attached to the apodemes of the extensor tibiae and flexor tibiae muscles. Zill (1985) was unable to confirm the presence of these elements in serially sectioned material. In addition to these distally running attachments, the body of the mtFCO possesses a ventrally located ligament that runs proximally to the flexor tibiae muscle (Usherwood *et al.* 1968; Matheson and Field, 1990).

Materials and methods

Specimens of adult *Schistocerca gregaria* (Forskål) of both sexes were obtained from a laboratory culture. For studies of internal anatomy, the metathoracic legs were removed and preserved. The anatomy was studied by several techniques. These included dissection of unfixed legs and legs fixed in alcoholic Bouin's solution, the preparation of whole mounts stained with osmium tetroxide, serial sectioning of resin-embedded legs for light microscopy, transmission (TEM) and scanning (SEM) electron microscopy. To prepare whole mounts, legs were removed and kept in a refrigerator for up to 24 h. Proximal regions of the femur and distal parts of the tibia were removed prior to fixation. Once fixation had commenced, parts of the integument overlying the chordotonal organ were cut away from anterior and posterior sides of femur. Legs were prefixed in Karnovsky's (1965) solution (0.5 h; 4.0 °C) and postfixed in osmium tetroxide until the mtFCO was stained brown. In many cases during prefixation, the tibia was held in

either the extended or flexed position using a short length of plastic tubing into which the leg was placed. In other cases the tibia was not restrained. Such preparations were dehydrated in acetone and mounted in EM-grade Araldite under coverslips supported by a plastic ring. For light microscope serial transverse sectioning, distal metathoracic legs were fixed in Karnovsky's (1965) fixative. Some cuticle was removed from the anterior face of each leg to facilitate penetration of fixative, solvents and resin. They were dehydrated in acetone and embedded in Araldite. Serial sections were cut at a thickness of either 1.0 or 1.5 μm; they were mounted in order and stained using either Toluidine Blue or aqueous Acid Fuchsin. Coverslips were mounted using EM-grade Araldite. Preparations for TEM were fixed and embedded in a similar way. They were sectioned and stained using lead citrate and uranyl acetate. A Siemens 102 TEM was used. For SEM, legs were dissected and prepared as for whole mounts; after fixation and dehydration, they were critical point dried and sputter coated. They were examined using a Cambridge S-100 microscope. Retrograde filling of mtFCO sensory cells with cobalt chloride was carried out following the method of Matheson and Field (1990). The preparations were left for 4–8 days for diffusion to take place, following which the stain was intensified (Bacon and Altman, 1977). Completed fills were fixed using either Carnoy or alcoholic Bouin. Changes in appearance of the mtFCO apodeme complex during passive extension and flexion in living, minimally dissected, unstained preparations were recorded photographically using phase-locked illumination. Photographs were taken at extreme phases of a 20° sinusoidal movement of the tibia about a mean 90° femoro-tibial angle. The technique was an adaptation of the one used by Stephen and Bennet-Clark (1982). The oscillator was an Exact model 337 digital phase generator. One output was used to drive a Bruel & Kjaer Minishaker (model 4810) which oscillated the tibia through an angle of $\pm 10°$ at any desired mean femoro-tibial angle. The other output from the oscillator was fed to a Neurolog NL403 delay width (to shape the light pulse) and a buffer power amplifier to drive the four LEDs used to illuminate the preparation once per cycle. A Watson Service II microscope fitted with a Nikon FE camera was used for all phase-locked photography. Because of the relatively low intensity of the LEDs, the photographs were a compromise between resolution, depth of focus, film speed and the number of cycles required to expose the film adequately. Exposures were taken with 100 ISO film over a 75 s exposure time, during which the tibia was oscillated at 2 Hz.

Results

General anatomy

The general arrangement of the organ and its attachments is summarised in Fig. 1. Distally, the organ is connected to proximal parts of the tibia by two structures: the flexor strand and the mtFCO apodeme complex. The flexor strand is attached to the distal region of the apodeme of the flexor tibiae muscle. The mtFCO apodeme is attached directly to the tibia just lateral to the insertion of the

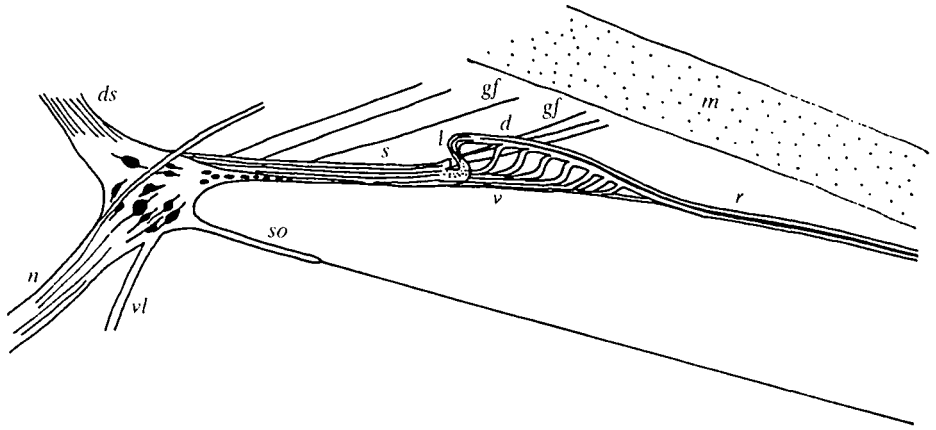

Fig. 1. This semi-schematic diagram shows some of the main anatomical features of the metathoracic femoral chordotonal organ (mtFCO) and the apodeme complex. A distal cuticular rod (*r*) is linked to the CO by dorsal (*d*) and ventral (*v*) ligaments. When the tibia is extended (as shown here), a loop (*l*) forms between them. A dorsal ligamentous strand (*s*) runs along the dorsal edge of the organ and is a continuation of the dorsal ligament. Just proximal to the loop, a number of guy-rope fibrils (*gf*) leave the strand to attach it to connective tissue elements within the leg. Just distal to the main guy-rope fibril attachment site, the dorsal ligament has a region in which the density of ligamentous fibrils is greatly reduced. Proximally, the organ is attached to the antero-dorsal femur wall by a well-developed continuation of the dorsal ligamentous strand (*ds*) and to the apodeme of the flexor tibiae muscle by a proximally running ventral ligament (*vl*). The position of the strand organ (*so*) is also shown for completeness. *m*, accessory extensor muscle; *n*, CO nerve.

apodeme of the extensor tibiae muscle (Field and Burrows, 1982). Distally, the mtFCO apodeme complex is a single epidermally secreted cuticular rod-like structure, but midway along its length it divides into two branches – one dorsal, one ventral – before joining the organ. A similar subdivision of the proximal apodeme complex into two has been noted in the chordotonal organ of the mesothoracic leg (Matheson and Field, 1990). The dorsal ligament consists of a continuation of the cuticular rod for much of its length, but proximally it is replaced by bundles of connective tissue cells. The ventral ligament is entirely composed of connective tissue cells throughout its length and there is no cuticular component. The flexor strand attachment is located ventral to the centre of rotation of the femur–tibia joint while the mtFCO apodeme is attached dorsal to it. Consequently, the flexor strand is stretched by extension of the tibia, while the mtFCO apodeme complex is placed under increased tension during flexion. The flexor strand appears to be very extensible and can take up the slack during extreme flexion. In contrast, the mtFCO apodeme appears to be stiffer and has been described as becoming 'buckled' under conditions of extreme extension (Field and Burrows, 1982). We have observed the behaviour of the apodeme and its associated ligaments during passive tibial extension in fresh preparations of

Table 1. *The average and range of femoro-tibial angles (degrees) at which the loop could definitely be seen in fresh unfixed preparations during flexion (last point at which the loop could be seen) and extension (first point at which the loop could be seen) of the left and right legs of three female and three male locusts*

	Females	Males	Mean, males and females
Flexion	37 (34–44)	35.8 (35–37)	36.4 (N=6)
Extension	50.2 (44–60)	52 (46–51)	51.1 (N=6)

minimally dissected legs. It is clear from such observations that buckling occurs over a considerable part of the range of joint movement and leads to the formation of a loop-like structure (Table 1). The loop appears at a level just distal to the proximal end of the dorsal ligament where the rod is completely replaced by ligamentous strands. Careful observation shows that the buckling affects only the dorsal ligament, producing the loop lateral to the ventral one. The latter remains unbuckled under all conditions of tibial extension and flexion (Figs 2, 4). The way in which the loop forms can be understood only in the light of more detailed anatomical studies. Sections of the distal apodeme show that it consists of a single flattened cuticular rod secreted by surrounding epidermal cells (Fig. 2D). Whole-mount preparations, SEMs and sectioned material show how this is replaced by the dorsal and ventral ligaments (Figs 2A,E,F, 3A–D). The two ligaments are distinct in their mode of origin and their structure.

The ventral ligament

The ventral ligament begins to form some 500 μm from the proximal end of the cuticular rod, where it is composed of a small number of fibres whose distal insertions are on the side of the rod (Fig. 2A,E). More proximally, additional fibres originating from the surface of the rod-containing dorsal ligament cross from it at fairly regular intervals to join the ventral ligament. Thus, the ventral ligament becomes thicker nearer to the organ as more fibres join it (Fig. 2A).

The individual fibres in the ventral ligament are composed of one to several elongate connective tissue cells (Fig. 3A,B). They have a dense cytoplasm packed with huge numbers of microtubules (Fig. 3C). The cells are surrounded by an amorphous extracellular matrix containing fibrils that stain pink with Acid Fuchsin in LM sections (Fig. 3D). Sections through the dorsal and ventral ligaments show that there is an ordered variation in the diameters of the fibrils according to their locations within the system. The largest ones are located on the outer surfaces of the ligaments. Further, there is a gradation in the diameters of these outer fibrils with the largest ones ($\pm$1.25 μm) on the ventral and lateral faces of the ventral ligament. Smaller ones ($\pm$0.5 μm) are found surrounding the connective tissue fibres in the proximal region of the dorsal ligament. In EM sections the stained fibrils are very electron dense with an amorphous appearance (Fig. 3D). They

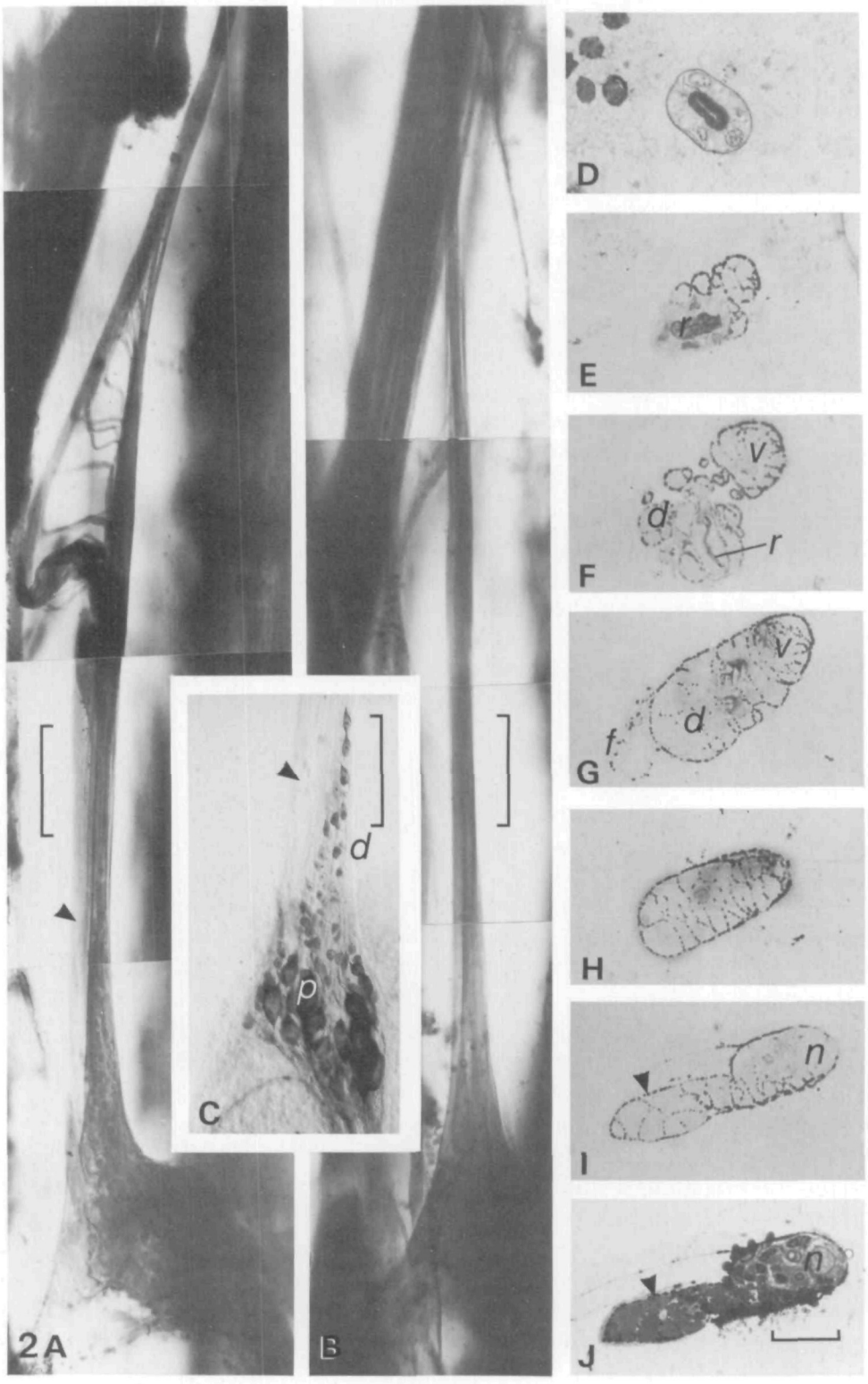
2A
B
C
d
p
D
E
r
F
v
d
r
G
v
d
f
H
I
n
J
n

Fig. 2. A and B show osmium-stained preparations of the organ and apodeme complex with the tibia in the extended (A) and flexed (B) positions. In A, the dorsal ligament has formed the loop and connecting fibres are visible running from the dorsal ligament to the ventral one. There is a gradation in lengths of the connecting fibres, with those most distal being the shortest. Proximal to the loop, the dorsal connective tissue ribbon (arrowhead) can be seen with the darker-staining ventral ligament and distal neurone region lying beneath it. In the fully flexed leg (B), the loop has straightened out. The dorsal connective tissue ribbon is distinct from the underlying ventral ligament. Retrograde fills (C) confirm the location of the distal neurone group (*d*) lying beneath the ribbon (arrowhead). The larger proximal neurones (*p*) are quite distinct. Sections of a flexed leg stained with Acid Fuchsin reveal the distal cuticular rod surrounded by epidermal cells (D) and its relationship to the ligaments (E–I). Visible in E is the insertion of the ventral ligament on the surface of the rod (*r*). In F, the end of the rod (*r*) and the origin of the dorsal ligament fibres (*d*) above the ventral ligament (*v*) are visible. G shows a section at the level of the origin of the main group of guy-rope fibrils (*f*) where dorsal (*d*) and ventral (*v*) ligaments have rejoined. The guy-rope fibrils stain in the same way as the fibrils surrounding the connective tissue fibres (visible in this section and others). At this level, the density of fibrils in the region corresponding to the dorsal ligament is greatly reduced. Proximal to the latter region, all fibres are once again surrounded by Acid-Fuchsin-staining fibrils (H). The fibrils are densest and of greatest diameter in ventral regions of the ligamentous region of the apodeme complex. I and J show the pattern of organisation at the level of the distal neurone group revealed by staining with Acid Fuchsin (I) and Toluidine Blue (J). They reveal the flattened dorsal ligamentous ribbon (arrowheads) and the position of the sensory neurones (*n*). Dorsal is to the left. In A–C, distal is to the top of the page. Scale bars: A–C, 200 μm; D–J, 20 μm.

resemble similar fibrils that have been seen associated with the neural lamella in distal parts of the tibia–tarsus chordotonal organ (CO) in the cockroach (Young, 1970). Although we cannot identify the material from which they are made, their amorphous appearance suggests that they are not composed of collagen. Previous descriptions of fibrils within the apodeme complex have suggested that they are composed of collagen, although there is no evidence that this is the case (Matheson and Field, 1990).

Since each cell is surrounded by fibrils, it is possible to follow the course of individual cells or groups of cells in serial semi-thin sections stained with Acid Fuchsin (see Fig. 2E–I). From such an analysis, we have established that the cells maintain a relatively constant position with respect to their neighbours and that their overall position within the ligament is very constant. In the ventral ligament, those cells that originate from the rod most distally are always found in its most ventral region. Conversely, those fibres originating from more proximal regions of the rod are located in more dorsal parts of the ligament. Fibres originating from intermediate positions on the apodeme occupy appropriately intermediate locations in the ligament. By tracing a considerable number of fibres it appears that this arrangement is strictly adhered to over their entire lengths. Many of these fibres could be traced as far as the region of the organ containing the scolopidia and small cell bodies of the distal neurone group (Fig. 2C). None of the fibres from

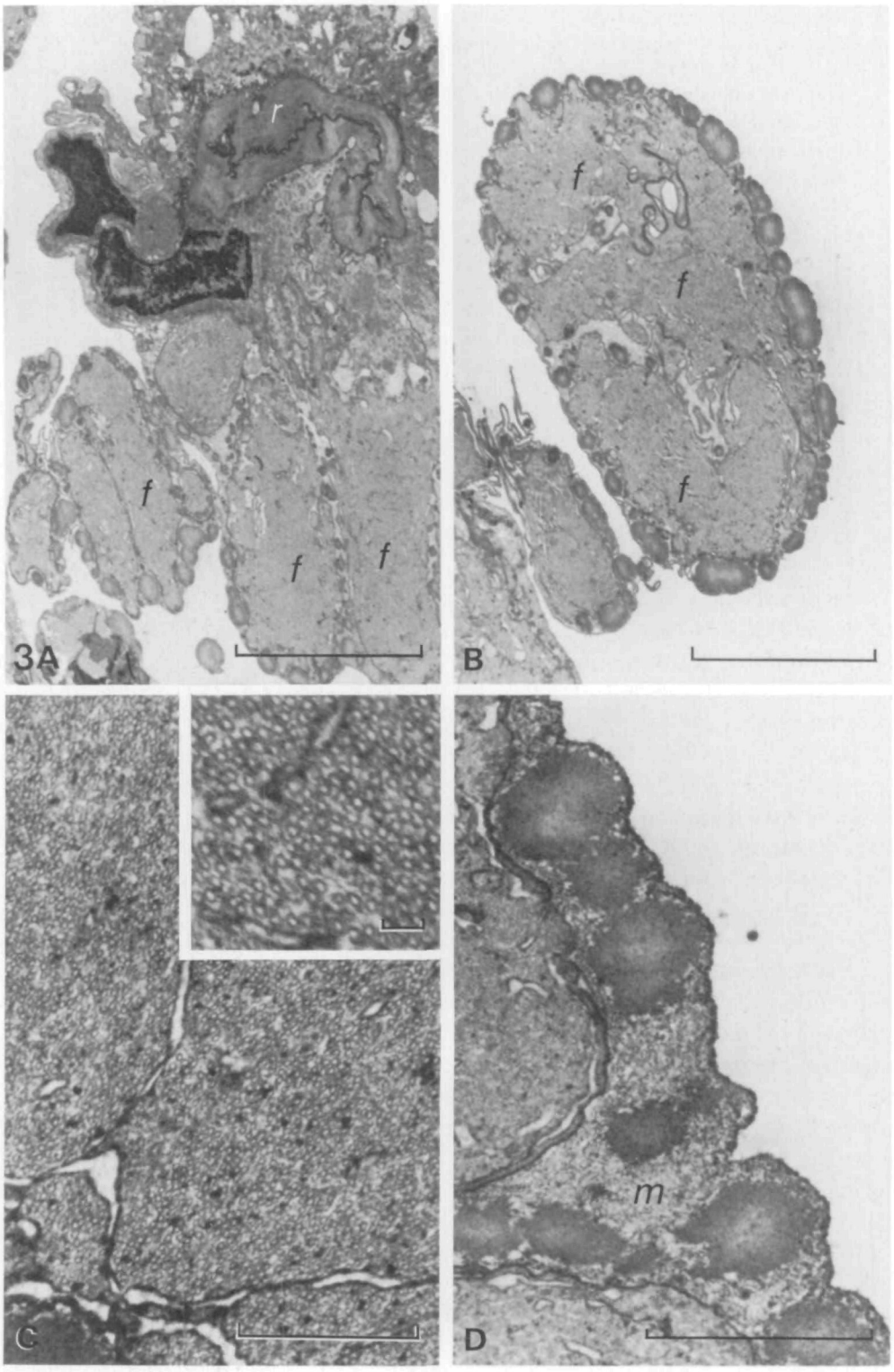
r
f
f
f
3A
f
f
f
B
C
m
D

Fig. 3. Electron micrographs reveal details of the three main structural components of the apodeme complex. (A) The cuticular rod (*r*) sectioned close to its end and a number of dorsal ligament connective tissue fibres (*f*) arising from their insertions on it. Some of the fibres consist of several cells. In B, the large fibre contains three such cells surrounded by densely staining fibrils. The cytoplasm of all connective tissue cells is packed with microtubules (C). The inset shows an enlarged portion of the micrograph. In D, the fibrils that stain with Acid Fuchsin under the LM can be seen to lie in an extracellular matrix (*m*) and to have an amorphous appearance. Scale bars: A, B, 5.0 μm; C, 0.5 μm; C inset, 0.05 μm; D, 1.0 μm.

the ventral ligament passes into the ligamentous ribbon that is clearly visible dorsal to the distal neurone group (Fig. 2A,C,I,J).

The dorsal ligament

The dorsal ligament arises as a direct continuation from the end of the cuticular rod, which forms its central core for most of its length (Figs 2F, 3A). However, at its most proximal end, the rod is replaced by bundles of connective tissue fibres of an identical type to those of the ventral ligament. The proximal destinations of the fibres arising from the end of the rod fall into three categories. Some of the most ventral fibres arising from the end of the rod join their neighbours from the ventral ligament and run with them into that part of the organ containing the small distal neurones. Slightly more dorsally, a few dorsal ligament fibres can be traced that run more dorsally towards the larger dorsal cell bodies of the proximal group of neurones (Fig. 2C). Finally, the most dorsal fibres within the dorsal ligament run as a bundle directly towards the dorsal part of the organ. However, most of them cannot be traced very far because, unlike the cells in other parts of the dorsal ligament, all these cells lose the majority of their surrounding fibrils over a 100 μm length. This region with greatly reduced numbers of fibrils is located just distal to a site of apparently considerable mechanical importance. It is here that the organ is attached to surrounding tissues by a series of guy-rope fibrils (Figs 2G, 4A,B).

The guy-rope fibrils

The main group of guy-rope fibrils is located at the distal end of a ligamentous ribbon that runs along the dorsal edge of that part of the organ containing the sensory neurones (Fig. 2A,C,I). Distally, this dorsal ribbon is of similar cross-sectional dimensions to the dorsal ligament. Proximally, it tapers and, in sections, the region previously occupied by the ribbon is seen to contain dorsal sensory neurones of the proximal group (Fig. 2C). Once again this ligamentous structure is composed of individual connective tissue cells each surrounded by a sheath of Acid-Fuchsin-staining fibrils (Fig. 2I). The ribbon is in direct line with those fibres of the dorsal ligament that contain reduced numbers of fibrils. However, the ribbon itself contains a full complement of fibrils at all points along its length.

Examination of the guy-rope fibrils in histological sections shows that they are indistinguishable in their Acid-Fuchsin-staining properties and dimensions from the fibrils surrounding each connective tissue cell (Fig. 2G). We conclude that they

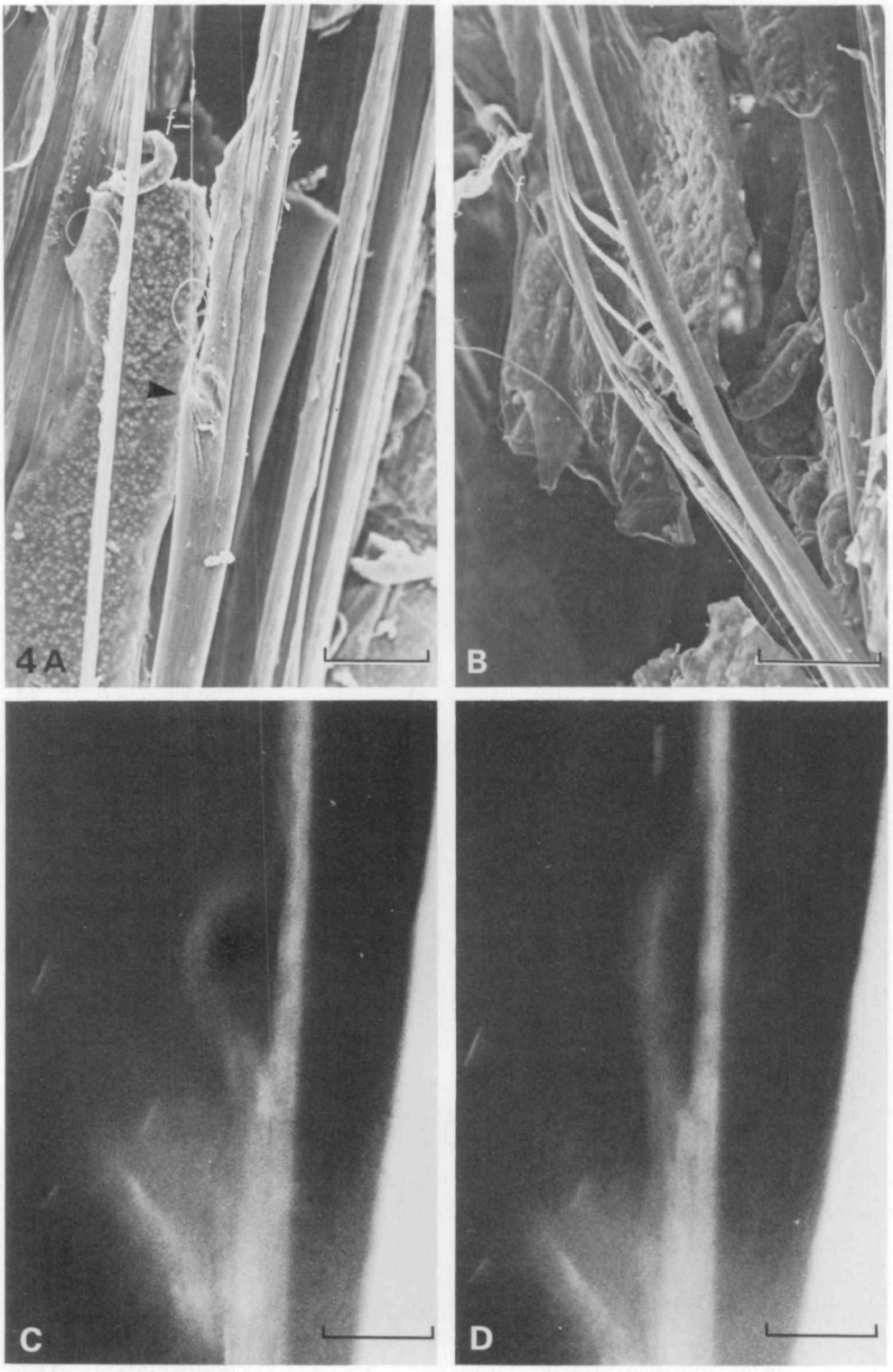
4A
B
C
D

Fig. 4. SEM preparations show the apodeme complex in the loop region with the tibia flexed (A) and extended (B). In the former case, the dorsal and ventral ligaments lie close together, in the latter they separate. In A, the attachment site of the guy-rope fibrils is visible (arrowhead) and a single guy-rope fibril can be seen (*f*). A similar fibril (*f*) is visible in the apodeme with extended tibia (B). The fibres running between the two ligaments are very clear in B. C and D show multiply exposed phase-locked photographs of a living preparation in which the tibia was sinusoidally oscillated ±10° with the tibia at a mean femoro-tibial angle of 90°. The photographs clearly show the characteristic changes in loop appearance with extension (C) and flexion (D). Scale bars: A–D, 100 μm.

are composed of the same material. They run in a distal direction and are joined to various connective tissue elements within the leg (Fig. 4A,B). We cannot exclude the possibility that some of them are attached to the cuticle directly but we have no evidence for that. In all preparations, whether flexed or extended, the guy-rope fibrils appear to be under tension because they are always straight and, where they join the main ligamentous ribbon, the tissue is pulled out in the direction of the fibrils (Fig. 4A). Something of their nature is revealed by examination of freshly dissected unfixed preparations using a mounted needle. Probing in the area of the fibrils shows them to be very taut and inextensible.

Formation of the loop

Under conditions of extreme flexion, the ventral ligament lies parallel to the apodeme and the dorsal ligament. In whole mounts, under these conditions, details of the double nature of the apodeme complex at this level can be resolved only by SEM examination (Fig. 4A). During extension, the rod-containing dorsal ligament moves proximally with respect to the ventral ligament so that it separates from the ventral ligament to form a loop. The dorsal ligament continues to be linked at intervals to the ventral ligament by the fibres that arise from its surface and cross to join the ventral one. These were traced in serial LM sections and they are clearly visible in SEM preparations (Fig. 4B). Whole mounts of the mtFCO complex fixed with the tibia in the extended position show that these crossing fibres are of graded length. Those nearest to the distal insertion of the ventral ligament on the rod are the shortest. Those nearest to the proximal end of the dorsal ligament are the longest (Fig. 2A). Consequently, when the tibia undergoes flexion from the extended position, the more distal linking fibres tighten before the more proximal ones.

To confirm that the loop forms under normal physiological conditions, the loop was photographed in minimally dissected, unfixed preparations during imposed sinusoidal movements of the tibia. These clearly show the opening and closing of the loop as the tibia is extended and flexed respectively (Fig. 4C,D).

Fibrils within the organ

The sensory cell bodies of the mtFCO lie in groups within discrete connective tissue pockets whose walls contain many similar Acid-Fuchsin-staining fibrils. In

addition to this connective tissue element, the more proximal regions of the organ are attached to a connective tissue membrane that is also richly supplied with fibrils and which is continuous with the dorso-posterior ligamentous attachment. The precise relationships of these fibril systems to those of the apodeme complex ligaments and the dorsal ribbon require further investigation.

Discussion

The above account shows that the mechanical link between the mtFCO and the femoro-tibial joint is much more complicated than previously thought. Its complex organisation must underlie some of the response properties of units recorded from the chordotonal organ itself and, until this linkage is better understood, no description of sensory neurone properties will be complete. So far, a number of conclusions can be reached. First, different groups of neurones in different parts of the organ appear to be connected to the joint in distinctive ways. There is a clear relationship between the ventral ligament fibres and the group of small distal neurones that was recently described by Matheson and Field (1990). These ventral fibres seem to remain under tension throughout the whole range of joint positions. In contrast, the dorsal ligament is slack over much of the possible range. It tightens up only when flexion results in joint angles of certainly less than 51° and probably less than 36°. In all cases where the loop was observed in fresh preparations, the smallest femoro-tibial angle at which the loop could be seen during flexion was always less than the angle at which the loop first became visible during extension. This difference may indicate some hysteresis in the system. However, the loop is extremely difficult to see in unstained preparations and part of the difference may be due to difficulties in deciding when the loop is present and when it is not. Until we have improved methods of illumination, this point cannot be decided for certain. Interestingly enough, the fibres that cross the loop from the dorsal to the ventral ligament provide a possible mechanism for range fractionation. Since they are of graded length, the most distal of these fibres must tighten up before the most proximal ones. We cannot escape the conclusion that this pattern of organisation is of functional importance. The formation of the loop requires that there must be compliant elements within the system. Based on our observations, it seems unlikely that the compliance is to be found in the cuticular or ligamentous components of the organ. Different types of insect cuticle show a wide range of mechanical properties (Neville, 1975) and some specialised types are very extensible (Vincent and Prentice, 1973; Andersen and Weis-Fogh, 1964), but our observations suggest that the cuticle rod of the distal apodeme and dorsal ligament is inextensible. Simple tests such as pulling on the rod with fine forceps reveal no evidence of extensibility. The fibres of both ligaments involve two components, which suggests that they are also very inextensible. These are the fibrils and the massive arrays of microtubules within the ligament cells. The former are composed of the same unknown material as the guy-rope fibrils. Direct examination shows the latter to be very inextensible. We do not think that the very

large bundles of microtubules are consistent with a structure that has inbuilt extensibility. If this were the case, loop formation could not be explained in terms of large differences in compliance between dorsal and ventral ligaments. Although the dorsal ligament contains a cuticular core over much of its length and the ventral ligament does not, larger differences in compliance of the two ligaments than are apparently found would be necessary to explain the formation of the loop. If that were so, regional differences in compliance must lie more proximally. Only direct observations of different parts of the organ during controlled movements of the tibia will be able to resolve these matters. Making such observations will be very difficult. Many of the components of the system are of extremely small dimensions and low contrast when unstained. This may explain why it is that no other workers have seen the structures that we have described, in spite of the fact that the mtFCO is one of the best-known insect proprioceptors. Further studies will require a detailed analysis of the mechanical properties of all the components in the system. We are currently developing methods that we hope will enable us to to do this. At this stage we can only speculate on these properties. In order to understand how the different components move relative to each other during joint movements, it will be necessary to carry out such observations on the mtFCO *in situ*, because the organ does not have a single, simple, proximal attachment to the cuticle of the leg. It is attached by ligaments both to the cuticle and to muscle. Therefore, it is likely that the whole organ moves relative to hard parts during joint movements. It is only by understanding all of these factors that we can begin to relate the physiological properties of identified mtFCO neurones to the mechanical properties of the organ. In the meantime, it is worth noting that many workers, when stimulating the mtFCO, cut the apodeme and hold it with fine forceps. Unless considerable care is taken to avoid damage to the loop-forming parts of the complex, such an approach is liable to generate spurious results.

We are grateful to Andrea Riley for technical assistance with certain aspects of the work. Parts of the study were supported by a grant to R.O.S. and P.M.J.S. from The Wellcome Foundation.

References

ANDERSEN, S. O. AND WEIS-FOGH, T. (1964). Resilin, a rubberlike protein in arthropod cuticle. *Adv. Insect Physiol.* **2**, 1–65.

BACON, J. P. AND ALTMAN, J. S. (1977). A silver intensification method for cobalt-filled neurones in wholemount preparations. *Brain Res.* **138**, 359–363.

BRÄUNIG, P. (1985). Strand receptors associated with the femoral chordotonal organ of locust legs. *J. exp. Biol.* **116**, 331–341.

FIELD, L. H. AND BURROWS, M. (1982). Reflex effects of the femoral chordotonal organ upon leg motor neurones of the locust. *J. exp. Biol.* **101**, 265–285.

KARNOVSKY, M. J. (1965). A formaldehyde–glutaraldehyde fixative of high osmolality for use in electron microscopy. *J. Cell Sci.* **27**, 137A–138A.

MATHESON, T. (1990). Responses and locations of neurones in the locust metathoracic femoral chordotonal organ. *J. comp. Physiol.* A **166**, 915–927.

MATHESON, T. AND FIELD, L. H. (1990). Innervation of the metathoracic femoral chordotonal organ of *Locusta migratoria*. *Cell Tissue Res.* **259**, 551–560.

NEVILLE, A. C. (1975). *Biology of the Arthropod Cuticle*. Berlin, Heidelberg, New York: Springer-Verlag.

STEPHEN, R. O. AND BENNET-CLARK, H. C. (1982). The anatomical and mechanical basis of stimulation and frequency analysis in the locust ear. *J. exp. Biol.* **99**, 279–314.

USHERWOOD, P. N. R., RUNION, H. I. AND CAMPBELL, J. I. (1968). Structure and physiology of a chordotonal organ in the locust leg. *J. exp. Biol.* **48**, 305–323.

VINCENT, J. F. V. AND PRENTICE, J. H. (1973). Rheological properties of the extensible intersegmental membrane of the adult female locust. *J. Mater. Sci.* **8**, 624–630.

YOUNG, D. (1970). The structure and function of a chordotonal organ in the cockroach leg. *Phil. Trans R. Soc. Lond. B* **256**, 401–428.

ZILL, S. N. (1985). Plasticity and proprioception in insects. I. Responses and cellular properties of individual receptors of the locust metathoracic femoral chordotonal organ. *J. exp. Biol.* **116**, 435–461.