

PROPRIOCEPTION IN *LIMULUS*

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(Received 17 May 1956)

(With Plate 9)

INTRODUCTION

Perception of the mechanical stimuli resulting from movement of Arthropod limbs has now been shown, in a number of cases, to involve special proprioceptive sensory endings at the joints. Apart from the internal organ found by Burke (1954) at the dactylopodite joint of the leg of *Carcinus*, these sense cells send peripheral processes which terminate in or directly under the cuticle, and the immediate stimulus appears to be the mechanical strain set up in the cuticle near the hinge by joint movement or by forces acting more distally on the leg. In Crustacea (Tonner, 1933) processes of the sense cells terminate without secondary cuticular modification in the joint membrane, but in insects (Pringle, 1938*a*) and in scorpions and *Phrynichus* among the Arachnida (Pringle, 1955) cuticular structures are associated with the endings in the form, respectively, of campaniform sensilla and lyriform organs, and serve to define more precisely the nature of the mechanical stimulus to which the endings respond. There are differences in structure between the campaniform sensilla of insects and the lyriform organs of Arachnids which indicate that these two types of sense organ have had a parallel rather than a consecutive evolutionary history, but it is probable that they serve an analogous function in these two major groups of terrestrial Arthropods.

The campaniform sensilla of insects and the lyriform organs of scorpions and *Phrynichus*, when suitably excited, initiate a discharge of impulses in their sensory nerves which shows slow and incomplete adaptation, and these endings can therefore provide continuous information about the position of the leg joints or the forces occurring during movements of the animal. In the cockroach many or all of the groups of campaniform sensilla on the legs are so situated that they signal the forces produced by contact of the leg with the ground rather than the position of the particular joint, and another type of sense organ, the hair plates (Pringle, 1938*b*), is probably the more important indicator of joint position. In scorpions movement of the joint is capable of eliciting a large sensory discharge even when the leg is out of contact with the ground, the impulse frequency in most of the fibres from the lyriform organ depending on joint position, with some acceleration of discharge during actual movement and if movement is resisted by an external object. In addition, there are present in the legs of the scorpion and *Phrynichus* sensory

endings with fibres of large diameter which are excited only by rapid movement of the joint in one direction and which adapt rapidly and completely when movement ceases. Certain characteristics of these discharges suggested (Pringle, 1955) that they did not originate from lyriform organs in the cuticle but from some form of internal ending at the joint whose nature was not then clear.

In a comparative survey of proprioception in the Arthropoda it is clearly important to include *Limulus*, an archaic, bottom-living, marine animal related to the stock from which the terrestrial Arachnida are descended. Opportunity to make a study of this sense in *Limulus* occurred during a short visit to the Woods Hole Marine Biological Laboratory in August 1955. After the experiments here described were completed it was discovered that Barber (1954) and Stuart (1953) had already interested themselves in this problem and some results have now been published by Barber (1956). Mr R. W. Stuart, whose histological work forms a valuable counterpart to the present experiments, has kindly allowed me to redescribe his observations in this paper and to publish some of his photographs of histological preparations.

MATERIAL AND METHODS

Large adult specimens of *Limulus polyphemus* (L.) were kept in the laboratory tanks and the legs removed as required by amputation across the base of the coxopodite. No differences were found between results from different legs.

Impulses were recorded in the leg nerves by means of wire electrodes, a Grass P 4 Pre-amplifier and a Grass Oscilloscope Camera.

To produce controlled mechanical movements, usually at the femoro-tibial (ischiopodite-meropodite) joint, the basal segments of the leg were firmly pinned to a wax block and the middle of a thread tied to the distal segment. One end of the thread was then attached by a length of elastic to a fixed support and the other wound on the shaft of a potentiometer controlling the Y-shift of the second beam of the oscilloscope. Approximately linear indication of joint movement is thus presented on the record.

RESULTS

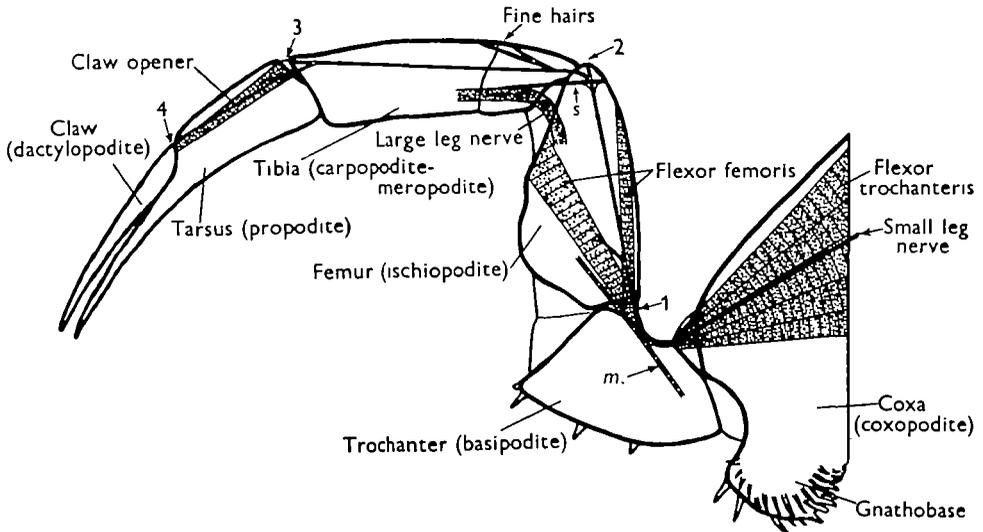
Anatomy and histology

Attention has been concentrated on the small leg nerve (the external pedal nerve of Hanstrom, 1928), whose field of innervation is shown in Text-fig. 1. This nerve arises independently from the ganglion ring and contains motor fibres to only three muscles, the flexor trochanteris in the coxa, the flexor femoris in the femur and the opener of the claw in the tarsus. The first two of these are levator muscles in normal locomotion and lift the leg off the ground; as in many Arachnids there is no levator muscle (extensor) at the femoro-tibial joint, this movement being brought about by a positive blood pressure and failing after excessive bleeding (cp. Ellis, 1944).

Only a small area of the leg surface on the outside of the tibia (near the line of fusion of the carpopodite and meropodite) has its tactile innervation in the small leg nerve. By far the largest and most noticeable sensory fibres arise from groups of

cells lying near the joint membranes at the trochantero-femoral, femoro-tibial, tibio-tarsal and claw joints (1, 2, 3, 4 in Text-fig. 1). These cells were first described by Stuart (1953), whose histological results are now quoted in detail. Similar cells at the coxo-trachanteral joint, which are innervated by one of the gnathobase branches of the main leg nerve, have been described by Barber (1954); these have not been studied in the present investigation.

Stuart's results were obtained with methylene-blue staining of the leg nerves. He found groups of sensory cell bodies under the median membranes of the first and second joints and under the lateral membranes of the third and fourth joints.



Text-fig. 1. The right third leg of *Limulus*, showing the field of innervation of the small leg nerve. 1, 2, 3, 4, location of proprioceptive endings; m., special muscle (see text); s., connective tissue strand.

The cells are typically multipolar with a single axon leading to the nerve and branching processes ramifying peripherally into the hypodermal cell layer under the joint membrane (Pl. 9, i, ii, iii). The size of the cells varies from $30 \times 60 \mu$ to $45 \times 100 \mu$, with indications of two distinct classes, a larger and a smaller. Differences are regularly found between the size of the cells at different joints. Thus, the groups at the median inter-segmental membrane of the first (coxo-trochanteral) joint contains a group of ten to fifteen cells of about $40 \times 80-90 \mu$ and, more distally, another group of smaller cells about $30 \times 60 \mu$, with thinner peripheral processes. The cells at the second joint (trochantero-femoral; 1 in Text-fig. 1) form a single diffuse group all of the smaller class ($30 \times 60 \mu$). At the third joint (femoro-tibial; 2 in Text-fig. 1) a group of very large cells, $45 \times 90-100 \mu$, are found 'at some distance from the lateral intersegmental membrane on the sensory nerve innervating this membrane and close to the junction of this nerve branch with the external pedal nerve' (Pl. 9, i); there is also a more numerous group of smaller cells 'distributed throughout the hypodermal tissue of the third joint's lateral membrane'.

Dissections made during the present investigation revealed that there are two short branches of the small leg nerve innervating cells at the femoro-tibial joint. The small cells giving rise to these sensory axons lie in two groups on each side of the hinge line, one group being thus at the extreme distal end of the femur (ischio-podite) and the other at the extreme proximal end of the tibia (meropodite).

Stuart also found two closely adjacent groups of cells at the fourth joint (tibio-tarsal; 3 in Text-fig. 1) and his illustrations of these are reproduced in (Pl. 9, ii, iii). The cells of Pl. 9, iii belong to the smaller class, $30 \times 60 \mu$, with fine peripheral processes, and those of Pl. 9, ii to the larger class, $40 \times 80-90 \mu$, with thicker peripheral processes; the branching of the processes in the hypodermal cell layer is well displayed.

Physiological evidence was obtained in the present investigation for endings in the claw joint (4 in Text-fig. 1).

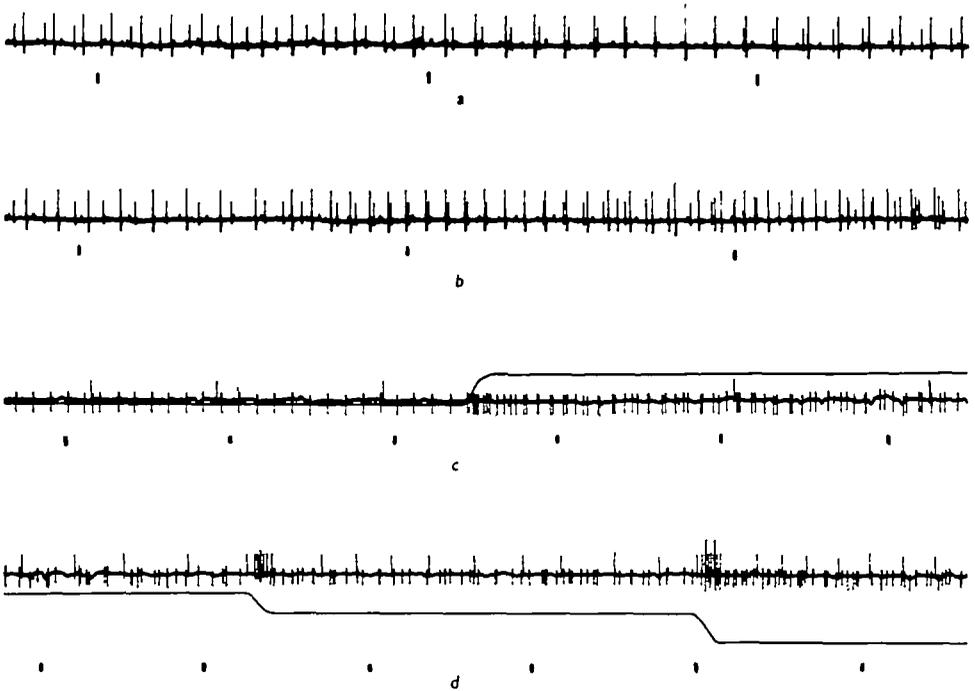
Physiology

Recordings have been made of impulses in the small leg nerve at two positions: in the nerve exposed in the femur and at the extreme base of the coxa. At the base, fibres are present sensitive to movement of the trochantero-femoral, femoro-tibial, tibio-tarsal and claw joints. The largest impulses were obtained from endings at the femoro-tibial joint and, since these appeared to be typical of the leg proprioceptors, they only were studied in detail.

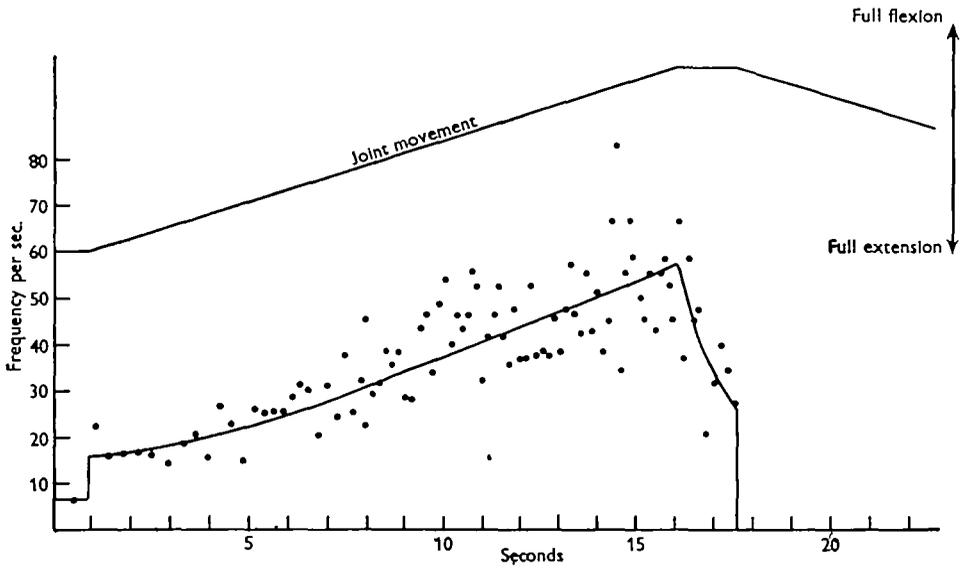
Text-fig. 2*a* shows the extremely regular discharge of impulses which may be obtained from endings at the femoro-tibial joint when this is held steady in a position of partial flexion. The frequency in these two fibres is, respectively, 10.7/sec. and 11.1/sec. and the discharge continues indefinitely. Allowing the joint to flex under the natural elasticity of the flexor muscles increased these frequencies to 13.9/sec. and 14.5/sec. and brought in a third fibre at 6/sec. (Text-fig. 2*b*); impulses in many smaller fibres were visible with high amplification.

There are present at the femoro-tibial joint tonic (incompletely adapting) endings whose discharge frequency increases on extension as well as endings excited by flexion. Text-fig. 2*c* shows such an ending responding to forced extension, in the middle range of joint movement, produced by the thread of the joint-position indicator; Text-fig. 2*d* shows two forced flexions in the same preparation. In Text-fig. 2*c* at the initial position of the joint an extensor fibre is discharging at 9.5/sec. and a larger flexor fibre at 1.15/sec.; at the final more extended position the extensor fibre, after an initial acceleration, is firing at 13/sec. and is joined by another of similar amplitude while the flexor fibre, after a short silence, returns at 0.85/sec. In Text-fig. 2*d* several flexor fibres are active and their frequency of discharge rises as flexion is increased; the largest of the tonic fibres shows considerable acceleration during movement and the second flexor movement initiates also two impulses from a phasic ending which has the largest sensory fibre in the whole nerve trunk.

A graphical plot of the response of the largest tonic flexor fibre to slow flexion is shown in Text-fig. 3. It is clear from the original record that many of the irregularities of frequency in this experiment resulted from failure to produce an exactly



Text-fig. 2. Oscillograms from *Limulus* small leg nerve, showing impulses in fibres from proprioceptor neurons at the femoro-tibial joint. *a*, joint partially flexed; *b*, joint allowed to flex fully under elastic pull of flexor muscle; *c*, nerve exposed in femur, discharge in tonic extensor fibres on forced extension, inhibition of tonic flexor fibre; *d*, nerve exposed in femur, discharge in flexor fibres on forced flexion, starting from more flexed position than record *c*. The second movement brings in a phasic fibre (retouched). In records *c* and *d* an upward movement of the joint-position indicator trace signifies extension. Time marker, 1 sec.



Text-fig. 3. 'Instantaneous frequency' plot of discharge in a tonic flexor fibre from femoro-tibial joint during slow joint movement. Each point is the reciprocal of the interval between one impulse and the next, and is referred to the instant of time mid-way between them. This ending was initially firing at 7/sec.

constant velocity of joint movement with the hand-operated control knob of the potentiometer (see Methods). This is the same flexor fibre whose response to more rapid movements is shown in Text-fig. 2*c, d*; the complete silence during even slow extensor movement shows that there is a considerable phasic influence in the excitation of this ending.

The overall picture of the functioning of this group of sensory endings is that there is a population of cells covering with both tonic and phasic responses the whole range of movement of the femoro-tibial joint. In general, the largest fibres show a phasic response and the smaller fibres pure tonic responses; those of intermediate size give an incompletely adapting discharge at a constant position of the joint but show considerable acceleration during movement in one direction and a silent period during movement in the other. Any given tonic fibre has a continuous range of excitation with a maximum at full flexion or extension; the minimum discharge from the whole group of cells is thus at about the mid-position of the joint (Text-fig. 2*a*).

IDENTIFICATION OF CELL POSITION

With the base of the small leg nerve on the electrodes the whole of the flexor tibialis muscle complex in the femur can be removed without abolishing the tonic response to joint movement; the phasic responses are usually absent after this amount of dissection. By careful section of the mid-line of the femoro-tibial hinge it is possible to cut the nerve branches to the tibia without destroying the femoral cells. Such a preparation shows fewer active endings but these include both extensor and flexor fibres. It appears, therefore, that there is no correlation between the location of the smaller cells and the sense of the tonic responses.

External probing with a blunt needle in the hinge membrane excites many of the smaller endings but never brings in the large phasic fibres. By contrast, internal paring of the membrane and adjacent regions of the femur and tibia with a sharp scalpel abolishes all tonic responses but leaves some of the large fibres responsive to rapid joint movement. These results may be correlated with Stuart's observation that the largest cells at the femoro-tibial joint are located far from the membrane surface at the junction of the joint branch with the small leg nerve. At this point the nerve is also joined to a peculiar strand of connective tissue (*s.*, Text-fig. 1) which in dissections resembles a nerve but on histological examination is found not to contain nerve fibres; neither is it a blood vessel, for in arterially injected specimens the small nerve is seen to be supplied by its own small artery running along it from the trochanter and only there joining the main leg artery surrounding the large leg nerve (Milne Edwards, 1872). This connective tissue strand runs from a point on the outer surface of the femur some way from the hinge to the main leg nerve in the tibia where it joins the connective tissue surrounding this nerve-arterial trunk. It seems likely, as suggested by Stuart (1953), that the large cells situated remote from the joint membrane are responsible for the large phasic impulses excited by rapid movement and the small cells near the membrane for the tonic discharges. This correlation, taken with the anatomical picture, suggests further that the large cells are

excited not by any movement or forces at the joint itself but by the internal tensions set up by the movement in the connective tissue. The strand mentioned above is so placed that its length must change during flexion and extension of the joint, whereas the small nerve itself is always rather accurately located between points at the femoro-tibial and trochantero-femoral joints which do not move relative to one another when the segments flex and extend. Text-fig. 1 also shows, at the trochantero-femoral joint, a small, fine muscle with close striation which runs from trochanter to femur and is attached by connective tissue to the small nerve where this runs round the inner curve of the trochanter. It is hard to see that this muscle can have any function other than to hold the nerve in this region away from the cuticle of the trochanter at or very near to the position of zero stretch when this segment moves. Such an arrangement is well suited to provide a fixed reference line for sensory structures responding to movement through the pull of other connective tissue strands whose outer insertion is on moving parts. Possibly some degree of plasticity in the connective tissue is responsible for the phasic nature of the response of these internal endings.

In the scorpion and in *Phrynichus* Pringle (1955) noted that the phasic impulses in large fibres, which were recorded in nerves at the base of the leg under conditions of movement very similar to those here described for *Limulus*, appeared to arise from structures lying deeper in the leg than the lyriform organs responsible for the tonic discharges. Histological investigations were not made in the course of that research, but the course of the small nerve fibre is the same and a similar arrangement of cells may be present in these Arachnids.

THE ADEQUATE STIMULUS FOR THE TONIC RESPONSES

Stuart (1953) showed that the peripheral processes of the small sense cells 'ramify in or between the cells of the hypodermal layer, close to the cuticle'. Bearing in mind the close similarity between the response of these endings and that of the Arachnid lyriform organs the question naturally arises whether the adequate stimulus for the tonic responses is strain in the cuticle near the joint or tension produced in the internal tissues as has been deduced for the phasic responses. Barber (1954), in his study of the proprioceptive endings at the coxo-trochanteral joint, states that no sensilla were seen in the membrane itself, but that the hypodermal cells under which the sense cells lie are less pigmented than those on either side and that the area can therefore be seen through the cuticle from the outside. There is no external sign of sensilla at the femoro-tibial joint, but the possibility is not excluded that some of the finer peripheral processes of the sense cells may penetrate the deeper layers of the cuticle and there react to strains.

Several experiments were performed on the femoro-tibial joint to test this hypothesis, and the results may be summarized as follows:

(1) Recording from the base of the small nerve with the leg intact, the position of minimum discharge in tonic fibres is always with the joint more than half extended; that is, flexor fibres are more in evidence than extensor fibres.

(2) Cutting the distal insertions of the flexor tibialis muscle complex on the inner side of the joint without other damage to the femur shifts the minimum further over towards complete extension, so that little or no discharge in extensor fibres is obtained on full extension.

(3) Cutting off the tibia also shifts the minimum markedly towards extension.

(4) Opening the femur by removal of the lateral cuticle so as to expose the small leg nerve shifts the position of minimum discharge towards flexion, and extensor fibres are now readily excited.

(5) The femoro-tibial hinge is a very free joint and if the flexor muscle insertions are cut there is little elastic bias. Nevertheless, such elasticity as exists is altered by damage to the integrity of either femur or tibia. Thus, with the segments intact there is a slight elastic force tending to produce flexion and this is increased by damage to the tibia; on the other hand, preparations with the tibia intact and the femur opened have a slight extensor elasticity.

(6) In an intact preparation showing a considerable tonic discharge on full flexion, pressure with a blunt point on the outside of the hinge inhibits the discharge and if sufficiently strong brings in extensor fibres.

(7) An isolated joint preparation with femur and tibia completely cut through about 1 cm. from the joint leaving only the nerve intact showed a considerable extensor discharge on full extension. Pressure applied to the cuticle of the tibia so as to expand the section parallel to the hinge line now abolished the extensor discharge and the preparation then showed a large discharge on flexion.

(8) It was always clear in these experiments that the flexor and extensor discharges occurred in different nerve fibres.

It is difficult to explain these results except in terms of the changes in stress produced in the cuticle near the joint by alterations of the mechanical conditions. If the cuticle at the hinge in the intact leg is normally under a small stress due to opposing and nearly equal stresses transmitted by the structure of the femur and tibia, and if the peripheral processes of the sense cells are inserted in the cuticle in two ways so as to respond to strains in opposite sense, then it would be expected that damage to the femur or tibia would alter the natural strain and bias the endings positively or negatively, so changing the position for minimum discharge in the manner found.

A difference must here be noted between the tonic responses from the femoro-tibial joint and those described by Barber (1954) from the coxo-trochanteral joint. In the present series of experiments it was always found that any given ending increased its frequency of discharge steadily as extension or flexion (as the case might be) increased from the point at which the ending was first excited, and that motion had a differential effect (Text-fig. 3). Barber reports that at the coxo-trochanteral joint each tonic ending is excited only over a certain range of joint positions and ceases to fire impulses when the joint is in a position either more extended or more flexed than this range; and that motion in either direction accelerates the discharge. It is possible that this difference in behaviour of the endings at

the two joints indicates a real difference in mechanism and function, but without comparative studies in the same animal the difference does not warrant further discussion.

CONCLUSIONS AND DISCUSSION

It is concluded from these experiments, in agreement with Barber (1954), that there are two types of proprioceptive ending at the femoro-tibial joint of the *Limulus* leg, and that these correspond to the two sizes and locations of sensory cells described by Stuart (1953). Large cells situated at the point of branching of the small leg nerve send processes into an internal connective tissue strand which is stretched by extension of the tibia and give rise to rapidly adapting phasic discharges in large diameter nerve fibres in the small leg nerve. Smaller cells, situated near to the mid-line of the hinge under the outside cuticle of the joint, send processes through or between the hypodermal cells into the lower layers of the cuticle where they react to strains and give rise to slowly adapting tonic discharges; these endings are of two types, some being more excited in the flexed position of the joint and some in the extended position, owing to the different type of strain produced in the two positions.

This conclusion points to a homology between the small-cell endings of *Limulus* and the lyriform organs of Arachnids, and suggests that *Limulus* may represent a more primitive stage in the evolution of this type of sensory ending in which cuticular sensilla have not yet arisen by secondary modification of the cuticular products of hypodermal cells. Wigglesworth (1953) has shown that in the campaniform sensilla of insects the sense cell, trichogen cell, tormogen cell and accessory cell arise by differentiation of the grand-daughter cells produced by division of a single hypodermal cell. The origin of the Arachnid sensory cells has not been studied, but if they and the sensilla-forming cells are found similarly to have a common grand-parent the parallel evolution of these two groups of terrestrial Arthropod would be further exemplified. Studies of the histogenesis of the Stuart cells in *Limulus* would then be extremely interesting from an evolutionary point of view.

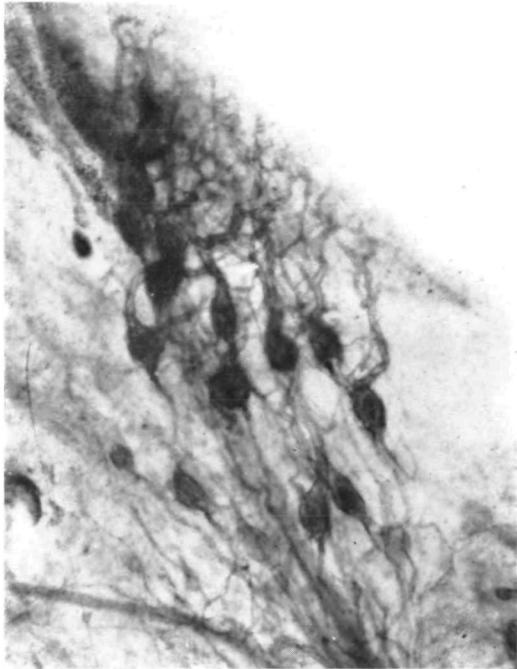
The large, internally situated, phasic cells cannot be compared directly with anything known in insects unless the chordotonal organs are found to have a similar ontogenesis. The phasic responses described by Pringle (1955) from a scorpion and *Phrynichus* may, however, arise from similarly placed cells. It is possible that the relatively greater reduction of connective tissue in insects as compared with Arachnids has precluded the evolution in the former group of this type of internal proprioceptive sense.

SUMMARY

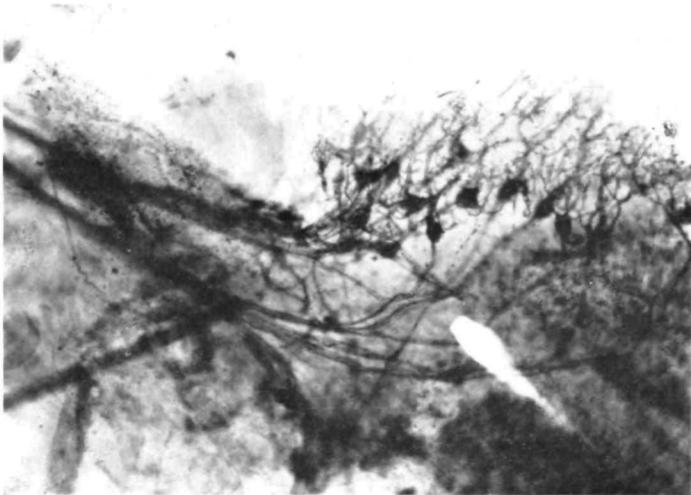
1. Tonic and phasic discharges of impulses may be recorded in the small leg nerve of *Limulus* from endings sensitive to the position and movement of the femoro-tibial and other joints.
2. The nerve fibres originate from the large sensory cells described by Stuart (1953). Of those at the femoro-tibial joint, one group of cells lies under the hinge



(i)



(ii)



(iii)

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(Facing p. 667)

and the endings react tonically to the strains present in the cuticle; some are excited by flexion and some by extension. The other group of cells, lying more internally, have endings in the connective tissue and react phasically only during joint movement.

3. The relationship is discussed between these endings and the lyriform organs of Arachnids and the campaniform sensilla of insects.

These experiments were conducted at the Marine Biological Laboratory, Woods Hole, during the tenure of Research Associateship of the University of California, Los Angeles. I am very grateful to Prof. T. H. Bullock for allowing me such free use of his apparatus.

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EXPLANATION OF PLATE 9

- (i) Group of large neurons located on the nerve branch innervating the femoro-tibial joint. Methylene blue; $\times 54$. (From Stuart, 1953.)
- (ii) Proprioceptor neurons from the tibio-tarsal joint; large cell group. Methylene blue; $\times 90$. (From Stuart, 1953.)
- (iii) Proprioceptor neurons from the tibio-tarsal joint; small cell group. Methylene blue; $\times 54$. (From Stuart, 1953.)