TWO NEWLY DESCRIBED SENSORY SYSTEMS IN DECAPOD CRUSTACEA : 1. THE CAMPANIFORM ORGAN SYSTEM. 2. THE CHEMORECEPTOR HAIR SYSTEM

R. G. J. Shelton

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Two Newly Described Sensory Systems in Decapod Crustacea.

I.The Campaniform Organ System.

II.The Chemoreceptor Hair System.

By

R.G.J.Shelton
Gatty Marine Laboratory
and Department of Natural History
University of St.Andrews.

Thesis submitted for the degree of Doctor of Philosophy.

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DECLARATION

I hereby declare that the work recorded in this Thesis has been carried out by myself, and that it is of my own composition. I further declare that it has not been submitted in any previous application for a higher degree.

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R.G.J.Shelton.

SUPERVISOR'S CERTIFICATE

I certify that Richard Graham John Shelton has fulfilled the conditions laid down in the regulations for a Degree of Doctor of Philosophy, under the ordinance No.I6 of the University Court of the University of St.Andrews and that he has accordingly qualified to submit this Thesis for the degree of Doctor of Philosophy.

M.S.Laverack.

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PREFACE

This study was commenced as a continuation of preliminary work by Laverack and later Cocup on what appeared to be a contact chemoreceptor system in the Decapoda. Methylene blue staining by Laverack of Panulirus argus walking legs revealed a complex of large, bipolar cells associated with the pad of epicuticle at the tip of each walking leg.

In the light of such knowledge as was then available, it seemed inescapable that these cells were part of a contact chemoreceptor system. The very large size of the cells was of especial interest and wholly exceptional for Crustacean chemoreceptors. Also, at that time the possibility of intracellular recording was an attractive one although we now know, from the work of Mellon & Kennedy (1964), that the impulse initiation site of Crustacean bipolar cells is not at the cell body but in the distal region of the dendrite. An intracellular study of these cells would not, therefore, have given any information about chemoreceptor transduction processes as had initially been hoped.

However, at this point the unexpected happened. Exhaustive tests failed to demonstrate the slightest chemical sensitivity in the large cell region and it was later discovered that these cells were associated with a rather specialized group of campaniform organs. This finding was, if anything, even more interesting than the initial premise upon which the project was based, since campaniform organs were then unknown in Crustacea. This thesis is an account of work carried out on these specialized campaniform organs and also their less specialized fellows which occur at other sites in Crustacean cuticle.

Another part of the study concerns the identification of the actual contact chemoreceptors of Decapoda. Here, electrophysiological methods were used as a histological tool to confirm anatomical observations. A number of types were discovered exhibiting, on the whole, considerable physiological similarity and equally considerable anatomical variation. The apparently high degree of correlation between structure and function was of no small interest and would in itself dispel any doubt that Decapoda are very highly developed animals.

INTRODUCTION

General Concepts of Smell and Taste.

Traditional distinctions between smell and taste, based as they are upon the specialized case of terrestrial vertebrates, are of but limited applicability to the case of aquatic arthropods. In terrestrial vertebrates this distinction is a clear one and in mammals at least, the taste buds and the olfactory epithelia have totally different response characteristics. These higher vertebrates, it would seem, have evolved a very considerable division of labour in their chemoreceptor population. The olfactory epithelium is differentially responsive to many of those substances of low vapour pressure which occur in the atmosphere and which may or may not be of some particular behavioural importance to the animal. The taste buds, by contrast, can only respond in four or possibly, in some desert mammals, five different ways to ions and a range of relatively soluble compounds. The flexibility of chemosensory response available to mammals is greatly increased by this taste/smell division of labour and because the olfactory epithelium is in communication with the buccal cavity, food in the mouth is still exposed to the full chemoreceptor population of the animal. We can begin to see, I think, that the distinction between smell and taste is as much a property of the animal as of the chemicals involved and that it is specialization of a high order indeed.

Because most early physiological knowledge was gleaned from the study of vertebrates and in particular of mammals, vertebrate - orientated thinking inevitably coloured some of the work of the early invertebrate physiologists. To what extent one can distinguish between taste and smell in aquatic Crustacea has been the concern of a number of workers. This study is merely the latest examination of this problem in a series commencing with the work of Cowles in 1908. However, before considering this problem specifically it is perhaps helpful to look at the general concept of chemoreception in a fully aquatic animal.

All chemoreception is ultimately aquatic to the extent that the odiferous substance is dissolved at least as early as the membrane of the receptor site. The essential difference between aquatic and terrestrial conditions in this respect lies in the media through which stimulatory chemicals have to travel from their source to the receptor site. Many substances which occur in effective concentration in air are relatively insoluble in water and conversely many substances which are freely soluble in water exert too low a vapour pressure to be available to the olfactory system of a terrestrial mammal. Many substances are in this latter class because their solubility in water depends upon their ability to enter the so-called "solvated" state in which each molecule of solute is surrounded by adherent water molecules. Trimethylamine oxide and amino acids are both in this class and are known to stimulate Crustacean chemoreceptors (Case & Gwilliam I96I, Laverack I963, Levandovsky & Hodgson I965). An aquatic animal is thus exposed to a large range of solvated substances which quite probably possess a certain degree of superficial similarity on account of their coating of water molecules but such an animal is denied contact with many other substances common in air but insoluble in water. It is this latter class of chemicals which are the especial concern of the olfactory membranes of mammals. The former class is available only to the sense of taste in a terrestrial animal but are available to the entire chemical sense of an aquatic one. It is apparent, then, that the chemical sense of an aquatic form can best be considered as a single entity and that it is futile to attempt to subdivide it into compartments based upon the traditional concepts of taste and smell.

It is clear, of course, that subdivisions of a rather different kind can be made which distinguish between a number of chemoreceptor types in Decapod Crustacea. However, these differences are based not, as in mammals, upon response characteristics but upon their position on the animal's surface and upon the part they play in certain chemically invoked reflexes. There is no evidence as yet that chemoreceptors from different parts of the body surface of Crustacea differ significantly in their response to stimulation.

Chemoreception in Decapod Crustacea.

The early workers in this field were concerned with 3 basic problems: (I) Do Crustacea possess a chemical sense?: (2) How is it used in the normal behaviour of the animal? and (3) What are the sense organs involved?

It may safely be stated that question (I) has been answered with an emphatic "Yes". Question (2) has been answered to the extent that chemical cues are known to play a large part in feeding behaviour, although many would still be prepared to dispute just how large this part is relative to the part played by the sense of sight. Question (3), on the other hand, has never been satisfactorily answered and until the advent of electrophysiological techniques there was little chance that any definite conclusions could be reached.

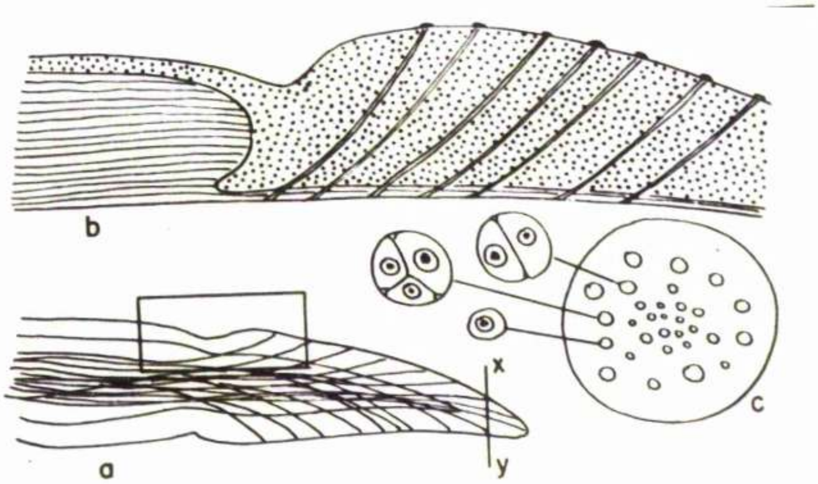
An important early paper was that by Nagel (I894) who emphasised the importance of the visual and tactile senses in the feeding behaviour of *Carcinus maenas*. Bethe (I897), however, was one of the earliest authors to recognise the importance of the chemical sense and like Nagel used *Carcinus* as his experimental animal. Bethe showed that the antennules exhibited increased activity when food was placed near the crab and that the maxillipeds would move slowly back and forth. His most significant observation was that painting the eyes did not prevent the crab from finding its food and that visual stimuli were not responsible for the observed activity in the antennules and the maxillipeds.

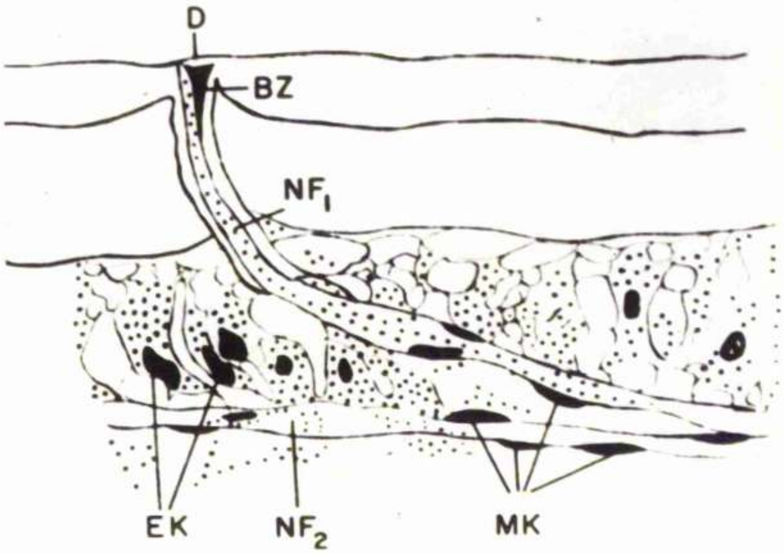
The work of Bell in I906 was an early attempt to study the location of chemoreceptor elements on the surface of the freshwater crayfish *Cambarus*. His method of localized stimulation by pipette was carried out under water and was only localized therefore for a very short time indeed. Bell's generally inconclusive results were therefore hardly surprising. He postulated a widely distributed system of chemoreceptors in which the antennules played no special part.

In his "funnel canal organ" Luther (1930) described a very unusual type of Crustacean sense organ. A widely distributed small sensory structure of this kind would have supported Bell's (1906) concept of a general chemical sense. Luther's suggestion that these sensillae were a kind of chemoreceptor we now know to be wrong but at least Luther was correct in describing these bodies as innervated structures. Dennell's (1960) description of what is clearly a group of funnel canals as cuticular glands is a case of mistaken identity which is only too easy in an animal with an integument as hard as Cancer pagurus. Diagrams by both these authors are the subject of Fig. (I).

Of particular interest to this present study is the fascinating observation by Cowles (1908) who described how the beach-living sand crab Ocypoda arenaria tests its food by first pinching it with the chela and introducing suitable torn-off pieces into the mouth. The chela sense organs involved in this behaviour are one of the topics covered in this thesis.

The ablation experiments of Holmes and Homuth in 1910 were an interesting early attempt to localize some of the chemoreceptors of Cambarus. Their results suggested strongly that the outer rami of the antennules were especially sensitive to chemicals. This was





<u>Fig. I</u>. <u>Early Interpretations of Crustacean Campaniform Organs</u>.

Above : Dennell's (I960) diagram of large diameter canal in <u>Cancer</u> walking legs. Canals are interpreted as cuticular glands.

Below : Luther's (I930) drawing of what he called a "Funnel Canal Organ" from a <u>Carcinus</u> walking leg. On morphologic grounds Luther suggested a chemoreceptor function for his Funnel Canal Organ.

confirmed electrophysiologically in 1964 by Laverack who was able to suggest with reasonable certainty that elements associated with the aesthetasc hairs were the chemoreceptors involved (Fig.2). This was in the large spiny lobster Panulirus argus . A chemoreceptor function for aesthetascs had been suggested as early as 1923 by Copeland.

As the first person to use electrophysiological techniques in the investigation of the chemoreceptors of Crustacea, Hodgson (1958) was also one of the few electrophysiologists to identify the actual chemosensory structures involved. His method of stimulating and recording from single, walking leg hairs using the same glass pipette was a very elegant and direct demonstration that hair sensillae were an important part and possibly the only part of the chemosensory system of the legs. Cambarus, as a freshwater Crustacean, was peculiarly suited to Hodgson's technique.

With the exception of Laverack (1964) most of the other electrophysiological work was concerned as much with the range of stimulatory chemicals as with the end organs mediating the response. The work of Case et Al. 1960, Case & Gwilliam 1961, Laverack 1963, Case 1964 and Levandovsky & Hodgson 1965 all falls into this class.

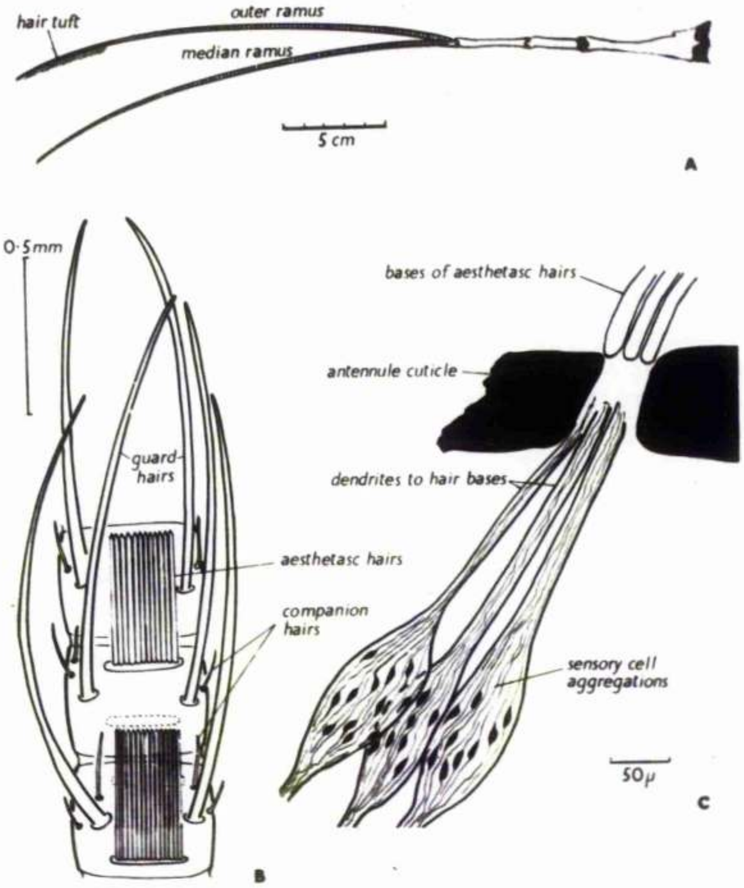


Fig. 2. A Chemoreceptor Hair System in *Panulirus argus*.

Laverack's (1964) drawing of the aesthetasc hair tufts present on the distal annuli of the outer ramus of the antennule. Subjected to a high degree of irrigation during antennule flicking, these untanned, unbranched hairs with multiple innervation are the simplest Crustacea chemoreceptor to be physiologically identified.

The enormous number of small neurones, 200-300, associated with the aesthetascs of _Panulirus argus_ was described in 1965 by Laverack and Ardill. This was one of the first indications that Crustacean chemoreceptor systems were different in some respects from their insect counterparts.

In this present work localized stimulation combined with electrophysiological recording from dissected nerve fibre bundles are the two main physiological techniques used in identifying some more Crustacean chemoreceptors and refinements of the same technique are used to specify the function of the so-called "funnel canal organ" which is here shown to be a mechanoreceptor with many features in common with the campaniform organ of insects. Conventional histological methods are used to complement the physiological experiments.

Mechanoreception.

In view of the reference to the mechanoreceptor function of the funnel canal organ it is perhaps apposite to say a little about mechanoreceptior in general and about the special mechanosensory problems encountered by an animal encased in a hard integument.

I would add that we are concerned here with the problems of mechanical communication between the cuticle and the outside world and not with the proprioceptive mechanisms associated with articulating segments of the integument.

In the case of soft-skinned animals pressure on the outer surface leads to considerable deformation of peripheral tissues and this deformation is itself an adequate stimulus for mechanically sensitive units, not only at the surface but also several millimetres deeper. However, because externally applied pressure does not, on the whole, lead to much deformation of arthropod cuticle, the tactile sense of this class of animals is faced with special problems of amplification. Hair sensillae, while indicating the proximity of external objects, cannot provide a sense of touch over the general surface of the cuticle. In insects this tactile sense is provided by the campaniform organ system first studied physiologically by Pringle (I938,I&2).

Each campaniform organ is a miniature mechanical amplifier with two main components, a flexible disc which is deformed when the cuticle near it is touched and an innervated peg articulating with the disc. Considerable variation occurs in the structure of these organs and a range of them is illustrated in Fig.3. However, the means by which they achieve their amplification and at the same time translate a movement in one direction to an amplified movement at right angles to the applied direction is the same for all types and a brief general description of how they work follows.

The disc acts effectively as a simple lever. Because the disc articulates with the body cuticle at the circumference of the campaniform organ, this articulation forms the fulcrum of the lever system. Strain in the surrounding cuticle therefore leads to a magnified movement at the centre of the disc. The degree of magnification depends upon the size of the disc. The larger the disc, the longer the lever and therefore the greater the degree of magnification. The disc is never perfectly flat but is always a shallow bump with the greater curvature facing distally. This restricts movement in the disc to the distal side of the diameter line.

The results of this present study indicate that it is to this class of sense organs that the "funnel canal organ" of Luther (1930) properly belongs and that while contact chemoreceptors are a very

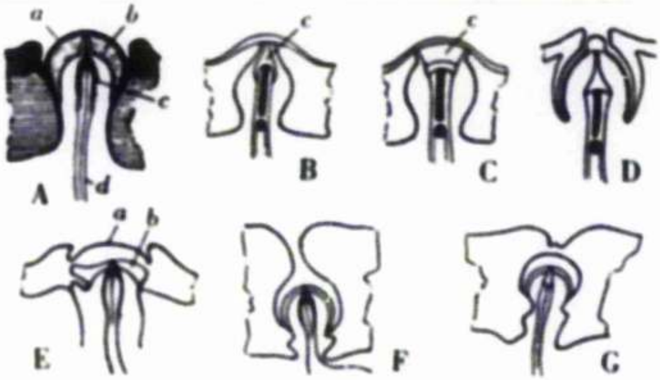


Fig. 9. Types of campaniform sensilla. A. Diagrammatic. B, C. Sections through the long and short diameters of a sensillum on the haltere of *Calliphora*. D. Sensillum on haltere of *Syrphus*. E. From the cercus of *Periplaneta*. F. From the labium of *Dytiscus*. G. From the mandible of *Dytiscus*. *a*, *b*, outer and inner lamellae of cap membrane; *c*, cuticular connexion of *d*, the distal process of the sense cell. (From various authors, after Snodgrass, 1935.)

Fig. 3. A Range of Insect Campaniform Organs.

First shown to be mechanoreceptors by Pringle (1938), this figure from his original paper illustrates the considerable diversity of structure shown by insect campaniform organs.

important part of the sensory equipment of Crustacea all existing physiological evidence suggests that the chemically sensitive neurones are associated with various categories of specialized hairs.

MATERIALS AND METHODS

Animals.

The Decapoda are a remarkably uniform group and this study was not restricted to any one species. Specimens of *Carcinus maenas* were obtained from Pitmilly and the Castle rocks area of St.Andrews bay. Common Lobsters, *Homarus vulgaris*, were obtained from a commercial fishery at Crail and also from St.Andrews bay. A few specimens of the Spiny Lobster, *Palinurus vulgaris*, were bought from W.Harvey, Fish Merchant of Penzance, Cornwall. All the above three species were kept in large tanks of circulated sea water in the Gatty aquarium.

In addition to this live material I also had access to a small number of *Panulirus argus* methylene blue preparations. Mortality was minimal in the case of the 2 species of lobster but tended to be high in the case of *Carcinus*, overcrowding in the aquarium being the main cause of this.

Histology.

The sensory innervation of the Funnel Canal Organs and other sensory structures was investigated by examination of fresh, live preparations stained with methylene blue (Gurr). Leg segments from all four species were split longitudinally with a scalpel and stained in 200cc. of chilled, filtered sea-water to which had been added I5 drops of a I% solution of methylene blue made up in filtered sea-water. Nerve cells were optimally stained after 30-45 mins. and could be examined in ice-cold, filtered sea-water up to an hour after staining.

Cuticular structures were examined in whole mounts dehydrated in ethyl alcohol, cleared in methyl benzoate and benzene, and also in paraffin sections of freshly moulted, soft-cuticled animals stained either with Heidenhain's iron haematoxylin or azan methods.

The principal problem associated with any histological operation involving the larger Crustacea is undoubtedly the toughness of the outermost layers of the cuticle. Consisting largely of tanned protein,this layer is largely unaffected by softening agents designed to render chitinized or calcified tissues sectionable. The epicuticle retains its durability even in freshly moulted animals and being protein tends to be hardened by fixatives. Over much of the body surface the epicuticle is quite thin and is no great problem to the histologist. However, in some places, notably at the tips of the walking legs and at those surfaces subject to abrasion the epicuticle is considerably thickened and here conventional histological methods are of little use.

In order to study the distribution of nerve cells below thick layers of epicuticle it is possible to sidestep the problem of hardness by removing the cuticle altogether. In the case of flat areas gentle stripping of soft from hard tissues is quite straightforward. In order to free the soft tissue at the end of the leg a little extra care is required to loosen the core of soft tissue which can then be withdrawn from the cuticle like removing a finger from a glove. For this part of the work these soft cores were embedded in paraffin wax and sectioned as above.

However, in order to study structures present in or passing through the epicuticle, hand sections of fresh material were useful for low resolution work while some success was achieved by embedding small pieces of the cuticle in hard "Araldite" resin and sectioning with a glass knife. Iμ sections obtained this way were then stained in toluidine blue. Attempts to prepare material for the electron microscope were rarely successful mainly because of the great difference in hardness between soft tissues, dendrites etc. and the epicuticle with which they were invested.

The only useful electron microscope results were obtained using *Homarus* material where the epicuticle is slightly softer.

It is perhaps worth adding that there is only one chemical mixture which will soften the tanned protein of the epicuticle and this is a mixture of glacial acetic acid and chlorine dioxide (Dennell 1960) available commercially as "Diaphanol" (Gurr). Softening takes a period of weeks and the effects of the mixture on soft tissues is quite disastrous.

Use of the Scanning Electron Microscope.

In many ways the Arthropoda are ideal subjects for the scanning electron microscope and in this study it was hoped to use it in the study of campaniform organs in Crustacea and also to examine chemosensory hairs in greater detail. Facilities were provided by the Cambridge Instrument Company and also by R.W.Ingle of the British

Museum (Natural History).

Specimens were fixed in Bouin's solution made up in sea water and then stored in alcohol. Then they were either slowly freeze dried or allowed to dry naturally in air. Both methods gave variable results with a slight bias in favour of freeze-drying. Prior to insertion in the microscope the specimens were vacuum-coated with Gold-Palladium.

The very fact that the specimens were from marine animals is a problem for scanning electron microscopy. Unlike most Insect cuticle that of marine Crustacea has a great tendency to contract and buckle during drying so that the interpretation of scanning pictures depends on basic information gained with the light microscope from specimens known to be free of contraction and flaking.

Electrophysiology.

Conventional methods of extracellular recording were used throughout. In all cases fibre bundles were subdivided using fine, tungsten needles and then picked up on a single platinum hook electrode leading to a differential A.C.-coupled amplifier and cathode ray oscilloscope (Tektronix 502A) display with audio monitor. Careful shielding of all leads combined with meticulous attention to earthing obviated the need for a "cage". Using this method it was possible to record from very small numbers of fibres supplying a small, known area of cuticle. The detailed arrangement of the preparations varied according to the type of sense organ being investigated and it is easier to discuss the various arrangements separately.

Chemoreceptor Study.

The main difficulty in working with chemoreceptor preparations lies in restricting the stimulatory chemicals to a known area and at all costs to protect exposed nerve fibres from the direct effect of the applied chemicals. In this work rubber diaphragms made from a child's balloon were used to separate exposed nerve fibres floating in saline from the rest of the preparation. Similar diaphragms were also used to restrict the flow of stimulatory droplets to known areas of cuticle. Fig.(4) shows a typical arrangement of this kind. Refinements of the method enable one to record from quite small numbers of chemosensory hairs. In order to fit the diaphragm, a pinhole is made in the rubber sheet and the piece of appendage is then pushed gently through (Case & Gwilliam I96I). Provided reasonable care is taken there are no

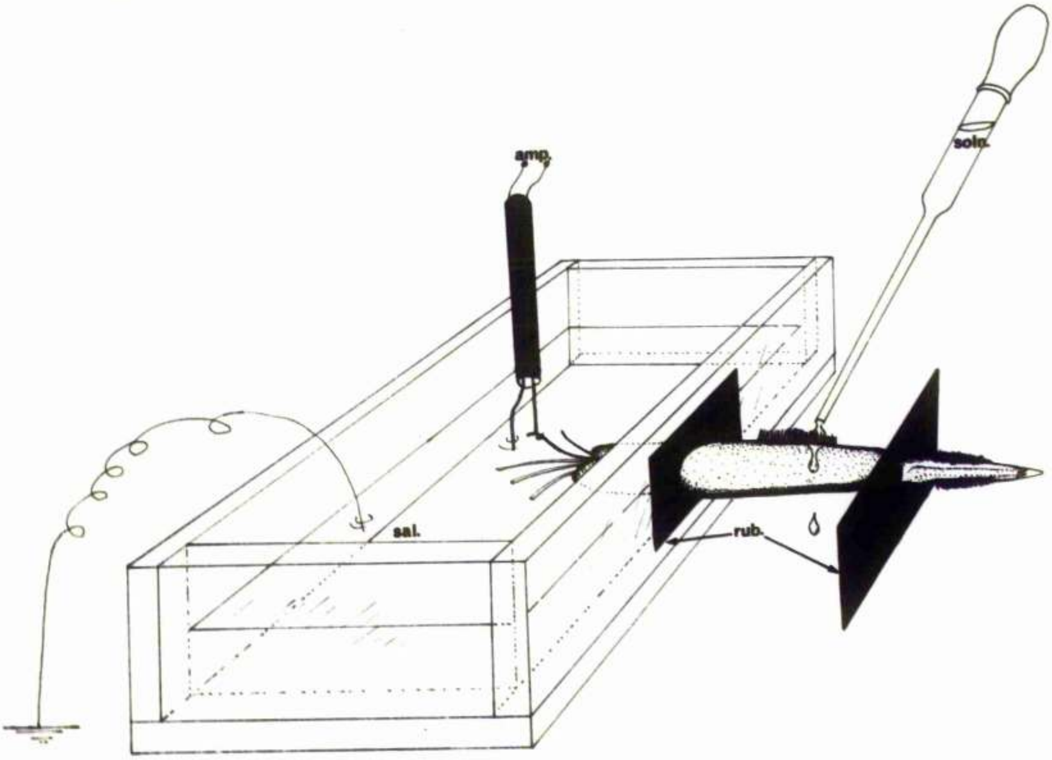


Fig.4. Experimental Arrangement for Recording from Chemoreceptor Hairs.

The preparation is severed from the animal and thrust through a sheet of rubber (rub.) which separates the chemoreceptors under investigation from the saline (sal.) in which the sensory nerve bundles are dissected. Individual bundles can then be connected to amplification equipment (amp.) by hook electrodes. Stimulatory chemicals (solu.) are applied to the preparation via a dropper.

problems of leakage. The precise chemicals to which Crustacean chemoreceptors are sensitive has been a subject of recent controversy (Case & Gwilliam I96I, Laverack 1963, Case I964) and since this was to be basically an anatomical study pure chemicals were rejected as the stimulatory substances in this work. All authors are agreed that the juices of most marine animals stimulate chemoreceptors in Decapoda and in this study *Mytilus* foot tissue and *Gadus* myotome tissue were used with equal success. In each case the solid material was ground in a pestle and mortar and left for a few minutes with the sea water in which the solid was later shaken up. Before being applied to the animal drop by drop the infusion was first filtered In the chemoreceptor study and indeed for all the electrophysiology cool (5°), filtered sea water was used as a physiological saline. Under these circumstances preparations would live for many hours. However, all experiments were completed within 40 minutes-I hour.

Mechanoreceptor Study.

Because all the experiments involved straining the cuticle, a reliable means of

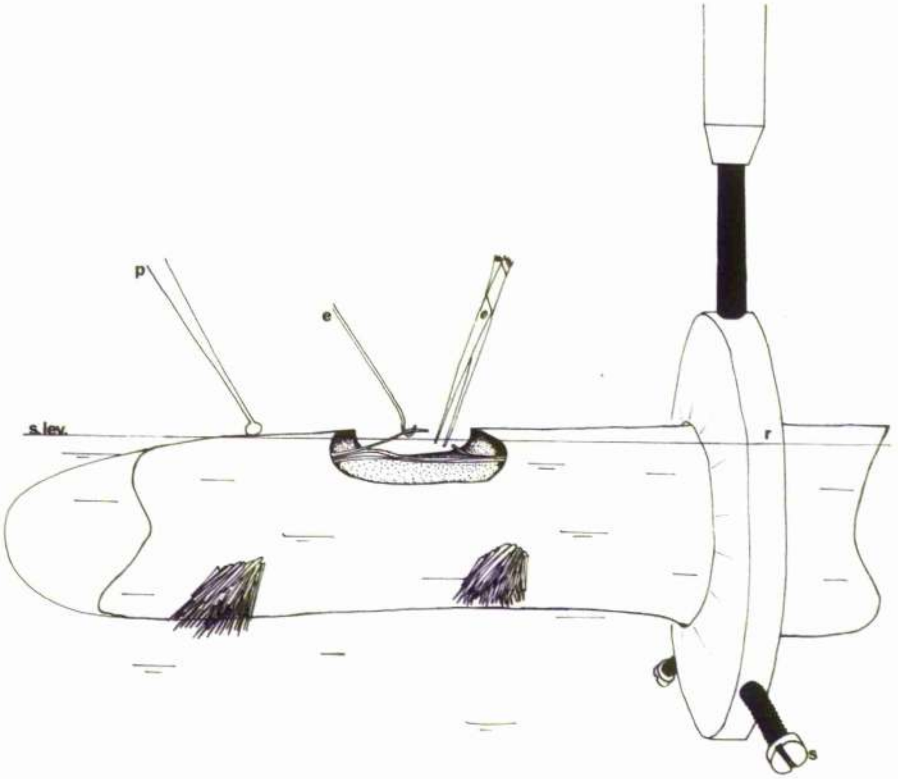


Fig.5. Arrangement for Holding Campaniform Organ Preparation.

For experiments with preparations of this type it is essential that the appendage or area of cuticle involved is firmly held and furthermore to take precautions to ensure that the method of holding the preparation does not stimulate the particular group of campaniform organs under investigation. This summary diagram illustrates a typical dactylopodite preparation set up for electrophysiological recording. The leg segment is held in a brass ring (r) by two screws (s). Strain is applied to the preparation by the probe (P) and the responses in the stimulated sensory nerves are recorded by the platinum hook electrode (e).

holding the preparation during stimulation was required and care had to be taken to avoid recording from units associated with that part of the cuticle which was in contact with the holding device. A number of devices were used and in all cases one or two screws were used as the main clamping agents. Fig.(5) shows a simple brass collar holder fitted with two screws which hold the preparation against the upper part of the collar.

A specially modified micro-manipulator (Prior) was used to apply strain to the cuticle and to hold it in set positions. This device is illustrated in Fig. (5). The movements of the manipulator are communicated to the animal via a stiff probe. A simple mechanical connection between the manipulator head and the spindle of a rheostat is the basic monitoring mechanism. A 6 volt battery is the power source and the variable output of this simple potentiometer is monitored on the screen of the cathode ray oscilloscope.

For low frequency phasic stimulation a Pen unit (Southern Instrument Company) was modified by replacing the pen writer with a stiff probe. This was driven by

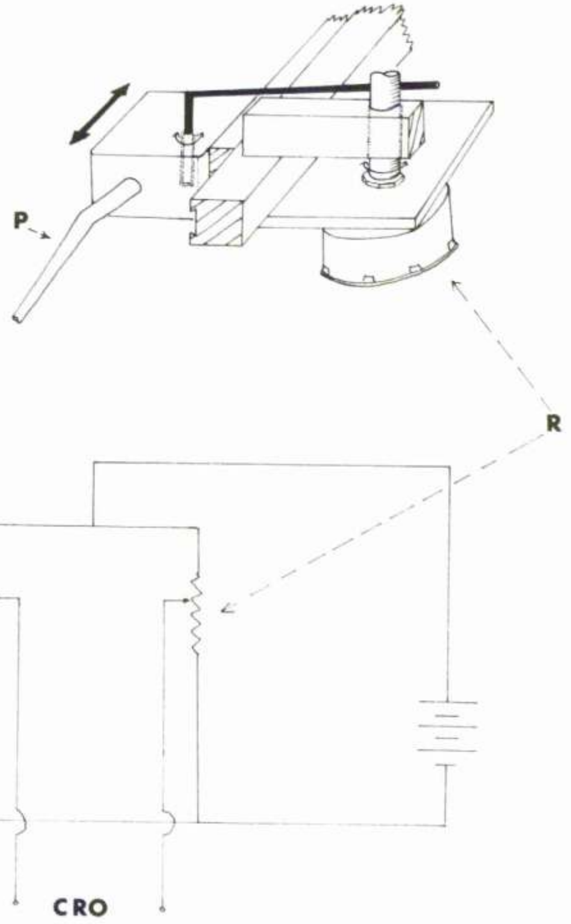


Fig.6. Simple Monitoring Device for a Prior Micromanipulator.

It is possible to monitor the movements of this type of manipulator, in the plane indicated by the double arrow, by connecting the head of the manipulator to a rheostat (R), by a simple mechanical linkage. Using the simple circuitry illustrated below it is then possible to monitor the movements of the probe (p) attached to the manipulator on the second beam of a cathode ray oscilloscope (CRO).

a U.L.F. oscillator (Dawe). Once again this was monitored on the second beam of the oscilloscope. For experiments involving stimulation at higher frequency (above 40 cycles per second), the brass probe was driven by a high frequency generator (Advance Instruments) via a 5 inch loudspeaker. The probe was connected to the cone of the loudspeaker as part of a second cone of sheet brass which was attached to the paper cone of the loudspeaker by "Araldite" resin adhesive (Fig.7). Monitoring of the stimulus on the second beam of the oscilloscope was achieved via leads direct from the generator.

Behavioural Techniques for Locating Chemoreceptors.

In addition to electrophysiological tests for chemoreceptor function various reflexes can be used as an indication that chemoreceptors have been stimulated. Nearly all appendages bearing chemoreceptors move after stimulation and the occurence of these reflexes after chemical stimulation at a known site is an effective way of identifying chemoreceptors. Leg retraction after chemical stimulation of the lower leg is illustrated in Fig. (8).

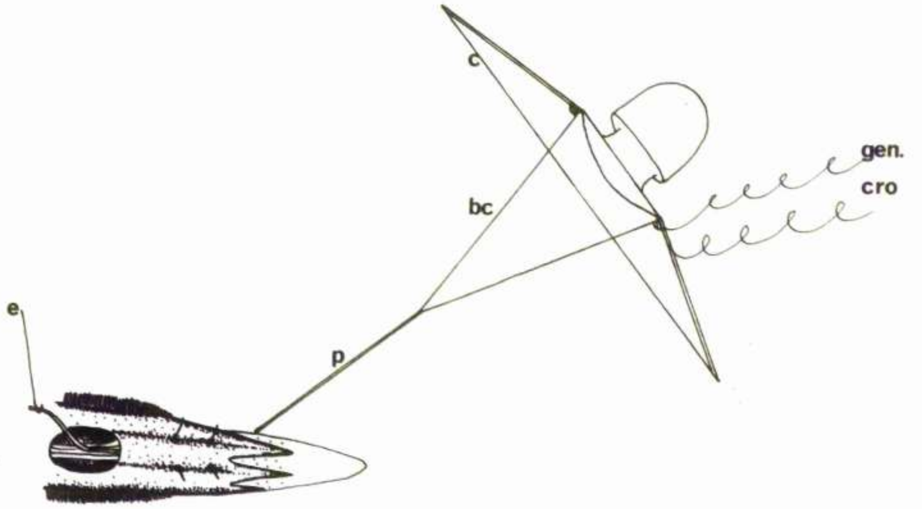


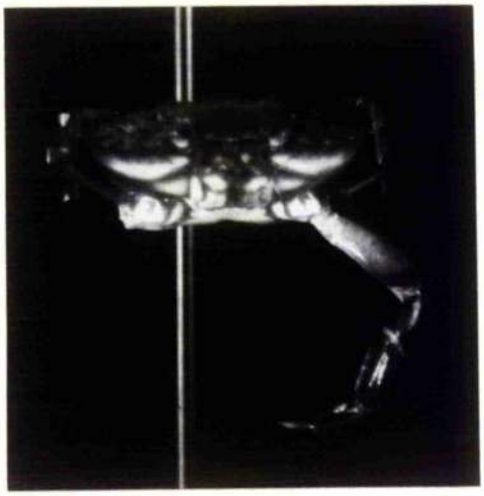
Fig.7. Modified Loudspeaker.

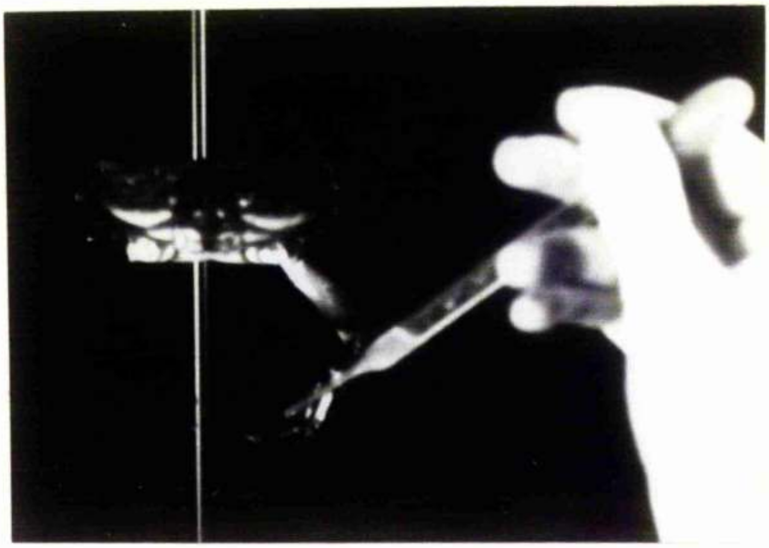
High frequency pulses are applied to the preparation from a modified loudspeaker driven by a generator (gen) and monitored on the second beam of a cathode ray oscilloscope (CRO).

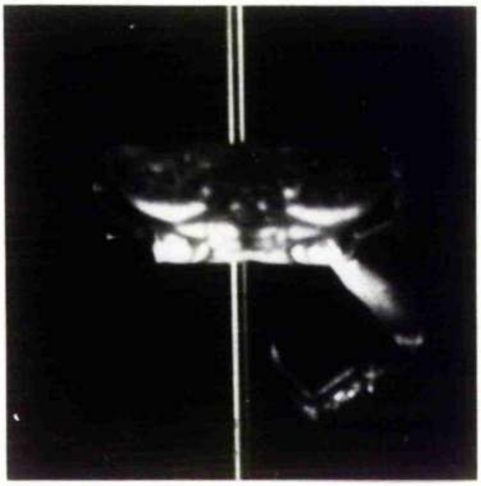
The probe (p) is attached to the cone (c) of the loudspeaker via a brass cone (bc).

Fig. 8. A Leg Retraction Reflex in *Carcinus*.

Chemical stimulation of the walking legs normally results in leg retraction and is a sensitive test for chemoreceptor function.







Simple Optical Models of Stress Lines in Cuticle.

The way in which campaniform organs are distributed over the surface of Crustacean cuticle is by mo means haphazard. The degree of stress existing in particular regions of the cuticle is certainly one of the more important factors correlated with the siting of the campaniform organs.

Pringle (I938,2) has discussed in some detail the types of stresses occurring in cuticle but this was not in itself sufficient to explain the anomalous distribution of campaniform organs on the surface of the upper leg segments and upon the carapace. Accordingly, Scott, who is currently working on the sensory input to the eye retraction reflex (Sandeman in press) and I secured the co-operation of Dr. Dhir of the Civil Engineering Department in the University of Dundee. We were interested in the way in which perforation of a lamina affects the distribution of stress lines in the stressed surface. Simple plastic models were used and the stresses set up were rendered visible by examining the models in a polarimeter where stress is manifested by multi-coloured fringes. Although it would be extraordinarily difficult to make a plastic model which exactly duplicated the behaviour of cuticle under stress the results obtained with the models provided a large part of the answer to the problem of anomalous distribution mentioned above.

RESULTS

Introductory.

It is extremely difficult, in a study of this kind, to separate anatomical and physiological results under separate heads without affecting the unity of what is essentially a piece of functional anatomy. However, despite this, the traditional distinction between anatomical and physiological information willbe retained in this account with the proviso that anatomical descriptions will tend to be couched in functional terms wherever physiological evidence exists to support them.

Anatomy.

An attempt to resolve the ambiguous situation left by the discrepancy between the results of Luther (1930) and Dennell (1960) formed the first part of this study. In essence it is a re-description of the main classes of cuticular structure to be found on the surface of Decapod walking legs.

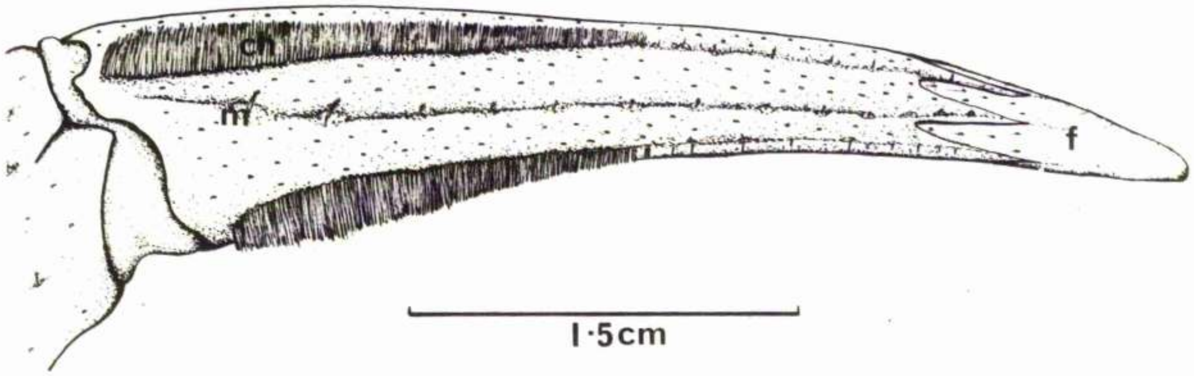
The terminal segment of the leg, the dactylopodite, contains no muscle and is essentially an elaborate battery of sensory and glandular structures borne on a thick cylinder of cuticle. The interior of the cylinder contains connective tissue, compartments of the haemocael, nerve cells and chromatophores. For most of its length, the cuticle in this segment shows a high degree of calcification and the epicuticle is greatly thickened and forms a relatively flexible pad at the end of the leg.

Fig. 9B represents the gross appearance of the dactylopodite segment of a walking leg of *Homarus vulgaris*. The epicuticular pad is visible as a rather translucent structure at the tip and hair sensillae are prominent in rows and bunches. Hair Peg Organs cover the surface but are absent from the terminal

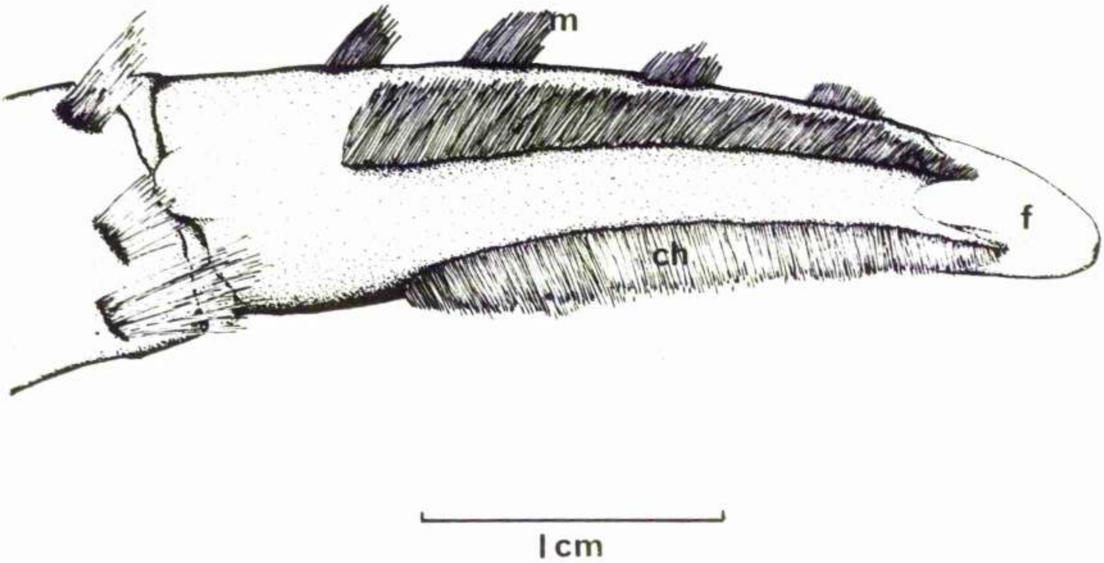
Fig. 9. Dactylopodites from Carcinus (A) and Homarus (B) Walking Legs.

As in all Decapoda and indeed most Crustacea and Insecta the epicuticle at the tip of each leg is thickened to form a flexible, durable pad (f.). The campaniform organs associated with this region are innervated by especially large neurones. Mechanoreceptor hairs (m) are present singly in Carcinus and in bunches in Homarus. In both cases chemoreceptor hairs (ch) are present in dense rows.

A



B



cap. The cap contains 2 types of cuticular specialization, Funnel Canal organs and the openings of cuticular gland ducts.

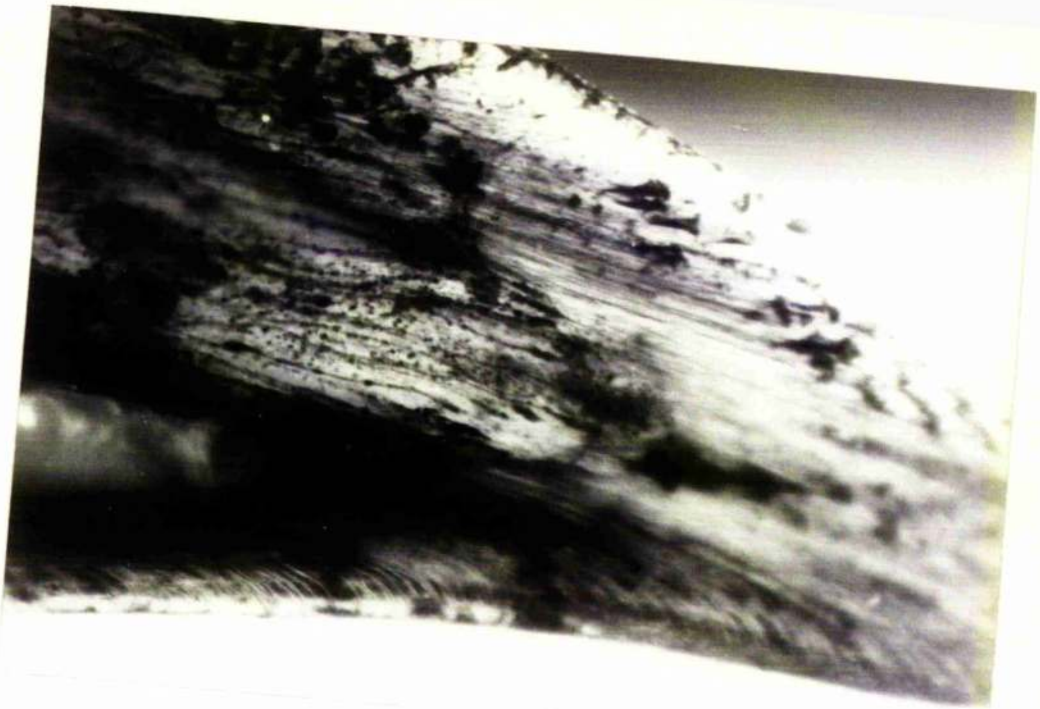
Fig.9A illustrates the equivalent segment of a Carcinus maenas walking leg. In this crab the epicuticular cap is a rather more complex structure. Tongues of thickened epicuticle extend proximally so that deep inroads are made into the hard, calcified cuticle of the proximal part of the segment. These tongues of epicuticle are held relatively rigid by the corresponding tongues of calcified cuticle with which they alternate. The portion of the epicuticular cap which is distal to the tongue region contains funnel canals and gland ducts only. However, the epicuticular tongues extending proximally from the tip contain funnel canals plus numerous "Buschelorganen" which occur over much of the surface of the cuticle and are the homologue of the Hair Peg Organs of Homarus (Laverack I962). Dense rows of hairs are present but large, single hairs take the place of the prominent bunches of Homarus.

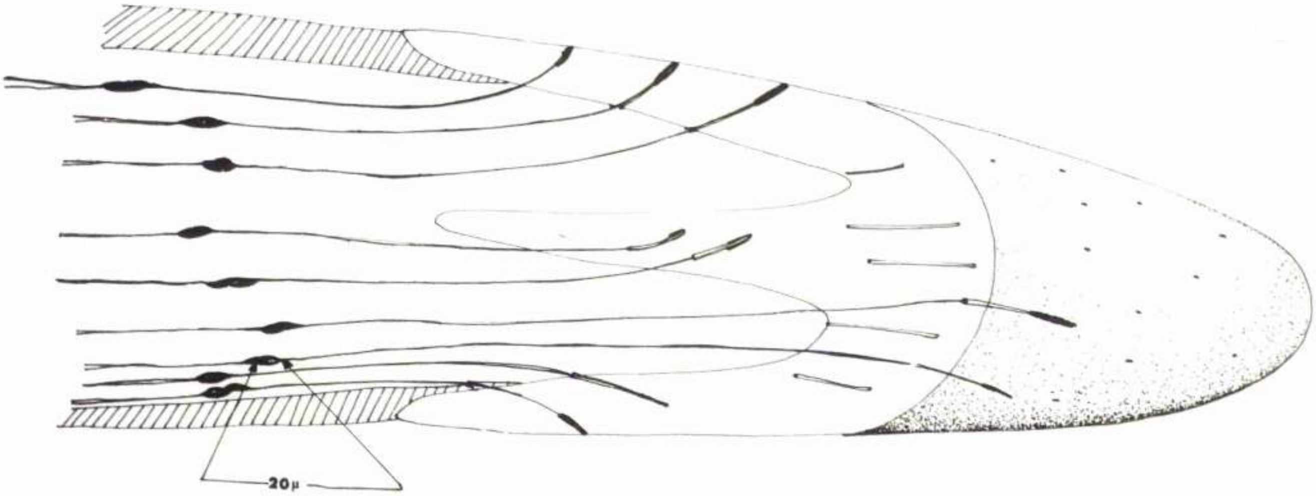
From evidence supplied by methylene blue preparations

Fig. 10. Sense Cells in Carcinus Leg Tip.

Above ; Methylene blue preparation of leg tip.

Below ; Summary diagram to illustrate campaniform organ innervation.




20μ

of the tip of the leg it was apparent that large neurones were associated with structures in this region, Fig.(I0). Closer examination of *Carcinus* and *Palinurus* preparations revealed that pairing between neurones was quite frequent and reminiscent of the results obtained by Whitear (I962) in her study of *Carcinus* leg proprioceptors. Similar pairing was seen by Taylor (I967) in a cuticular chordotonal organ in the antenna of a hermit crab (*Petrochirus californiensis*) and also by Wyse & Maynard (I965) in the antennule of *Panulirus argus*. However, in this latter instance there was a considerable distance between the 2 cell bodies in the pair. Pairing of neurones also occurs at the leg tip, Figs. II & I2 in *Palinurus*, *Carcinus* and *Homarus* and as in the cases cited above, there is always a definite disparity between the sizes of the two neurones forming the pair.

The number of pairs concentrated at the tip of the dactylopodite appears to be correlated with the hardness of the epicuticular pad at the end of the leg. Where

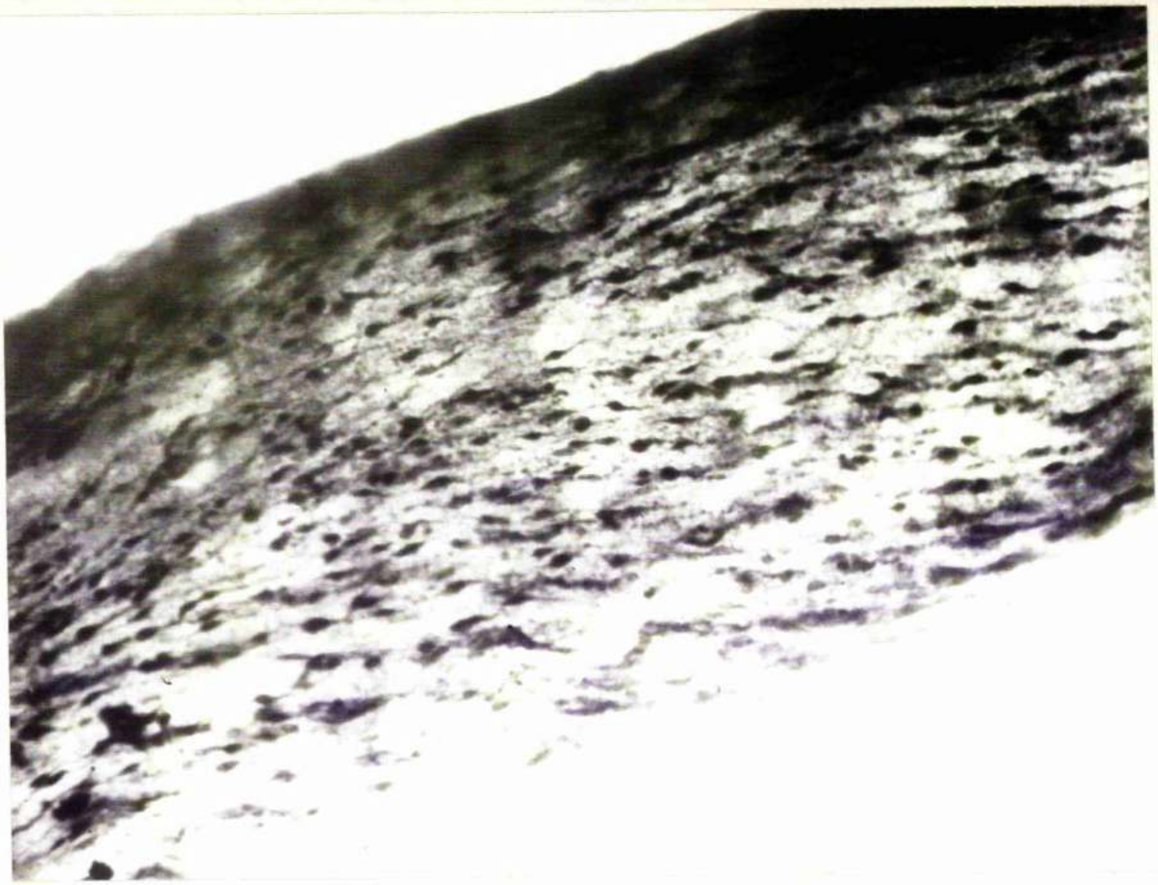
Fig. 11. Campaniform Organ Neurones from a *Palinurus vulgaris* Walking Leg.

In these whole mounts, the tip of the leg has been withdrawn from the cuticle and the resulting "pull-out" has been stained in Methylene Blue (Gurr).

A) The dense innervation of the tip region.

B) Pairing between campaniform organ nerve cells.

A



B

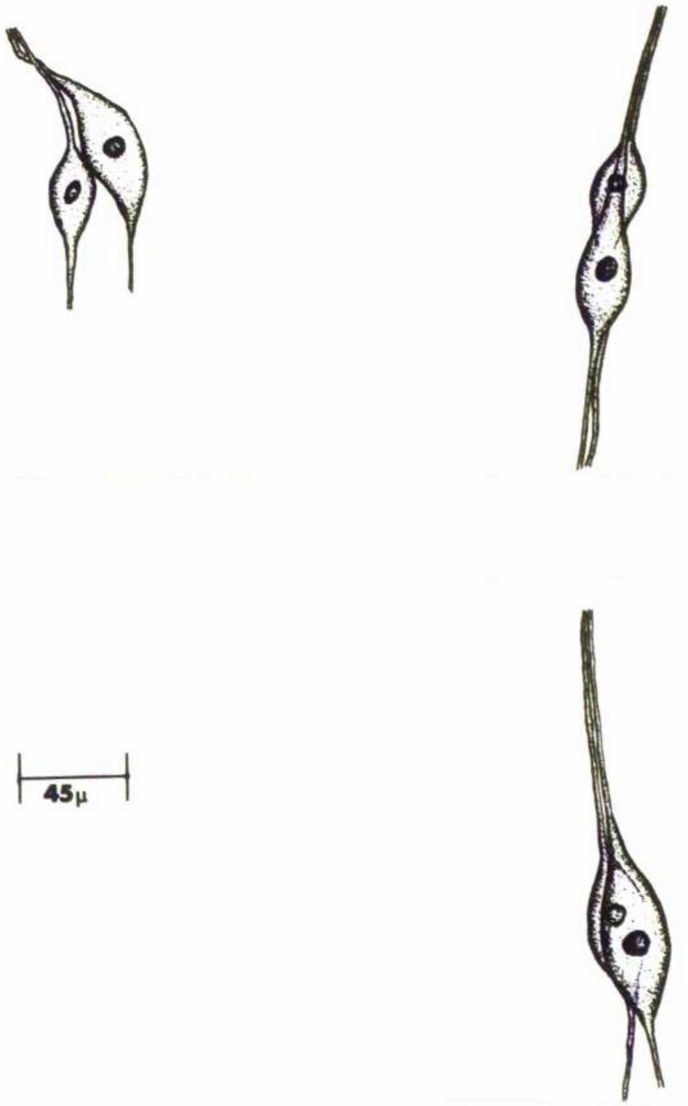


Fig. 12. Neurone Pairs Seen in Methylene Blue Preparations of *Palinurus* Campaniform Organs.

Pairing of this kind is characteristic of the campaniform organs at the leg tip.

this pad is soft, a relatively small number of pairs is present (6 to IO in *Homarus*). For *Carcinus* the figure is 90-120 and in *Cancer* and *Palinurus* the numbers are very much higher still and the cell bodies are very densely packed in the tip region (Fig.IIA and Figs.I3 & I4).

The comparative softness of the epicuticular pad in *Homarus* enabled transverse sections of the canals running through the pad to be cut thin enough for examination in the electron microscope. This demolished entirely the Dennell (I960) interpretation of the canals a s gland ducts. The canal consists of 2 concentric tubules separated by the cytoplasm of a hypodermal cell. Both inner and outer tubules consist of dense cuticle which stains strongly with acid fuchsin in light microscope sections. The inner tubule contains 2 dendrites and what appears to be part of a sheath cell. An oblique transverse section is shown at low and at higher magnification in Fig.I5. The inner tubule ends distally as a solid peg which is embedded in a delicate cap. This structure is essentially similar for *Homarus*, *Carcinus*, *Palinurus* and *Panulirus* and Fig.I6, while drawn from a *Carcinus*

Fig. 13. T.S. Leg Tip. Palinurus.

Large numbers of campaniform neurones (C) are packed into the tip region. H is a lacuna of the haemolymph system.


C
H
C

Fig.14-L.S. Leg Tip. Palinurus.

In this thin section of the soft tissue of the leg tip, large numbers of nerve cell bodies(C) of campaniform organ neurones are seen. Dendrites from these (seen with sheath at D) project into the cuticle of the leg tip.


D
C

Fig.I5. Oblique Transverse Section of the Dendrites of a Single Campaniform Organ in the Tip Region of a *Homarus* Dactylopodite.

This is an electron micrograph which illustrates the structure of the so-called "Funnel-Canal" of Luther (I930). It is essentially two concentric tubes of tanned cuticle which form the inner (i) and outer (o) cuticular sheaths of a pair of dendrites (d) and their sheath cell (s). The double tube runs through the flexible epicuticle (e) of the leg tip. Between the two tubes is the cytoplasm of a single hypodermal cell (h). The illustration on this page is at a magnification of xI3,500. On the succeeding page the dendrites and sheath are depicted at a magnification of x32,400.


d
d
s
o
h
e
X 13,500


l
d
d
s
l
X 32,400

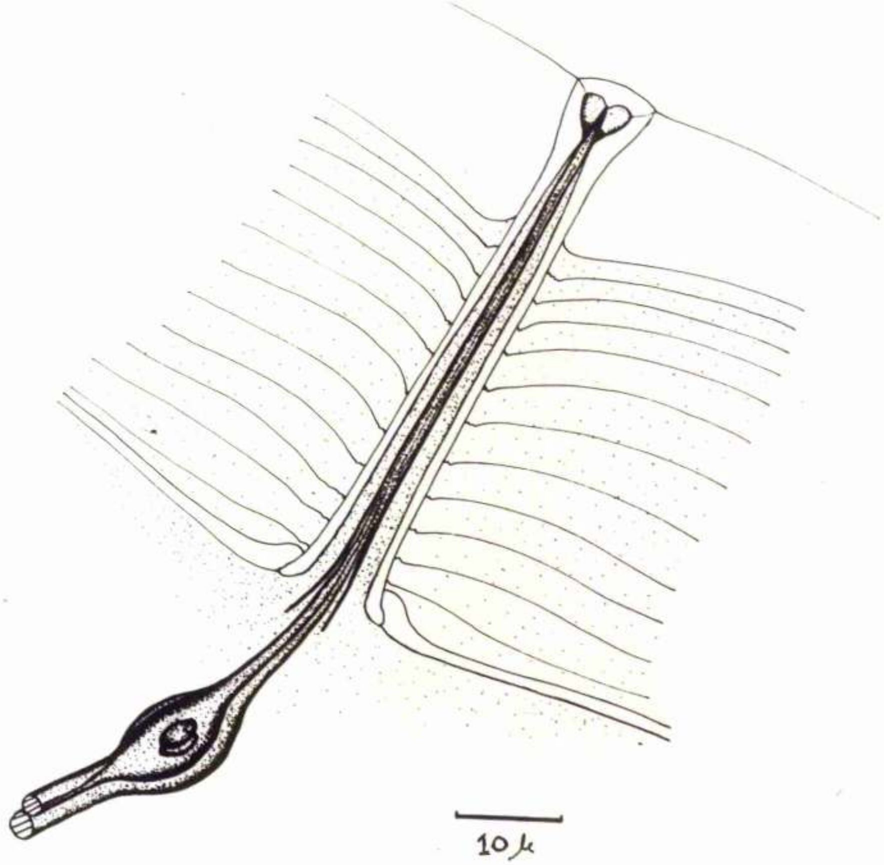


Fig. 16. Summary Diagram of Carcinus Campaniform Organ.

This shows the basic structure of a Crustacean campaniform organ with 2 or more neurones connected to a distal delicate cap.

preparation, represents a typical Crustacean campaniform organ. The cap of the campaniform organ is an extremely delicate structure and is in the form of a shallow dome (Fig.I7). It stains fairly strongly with methylene blue and exhibits a mild fluorescence under ultra-violet light. It thus appears to be cuticle of the same general type as the resilin found at hair bases (Thurm 1965) in insects.

The part of the cap into which the innervated peg is inserted varies with the type of campaniform organ involved. Usually, the insertion is central but where the cap is ellipsoidal in shape, as it is at the tip of the leg, the insertion is at the distal end of the ellipse.

Cuticular Glands.

As will by now be clear the conspicuous, long, curved canals associated with the end of the leg are part of an aggregation of what will later be shown to be a rather specialized kind of campaniform organ. However, cuticular glands are present in large numbers in the dactylopodite as globes of cells secreting into a single fine duct originating at the centre of the globe and proceeding distally to open at the surface. Fig.I9 shows a typical

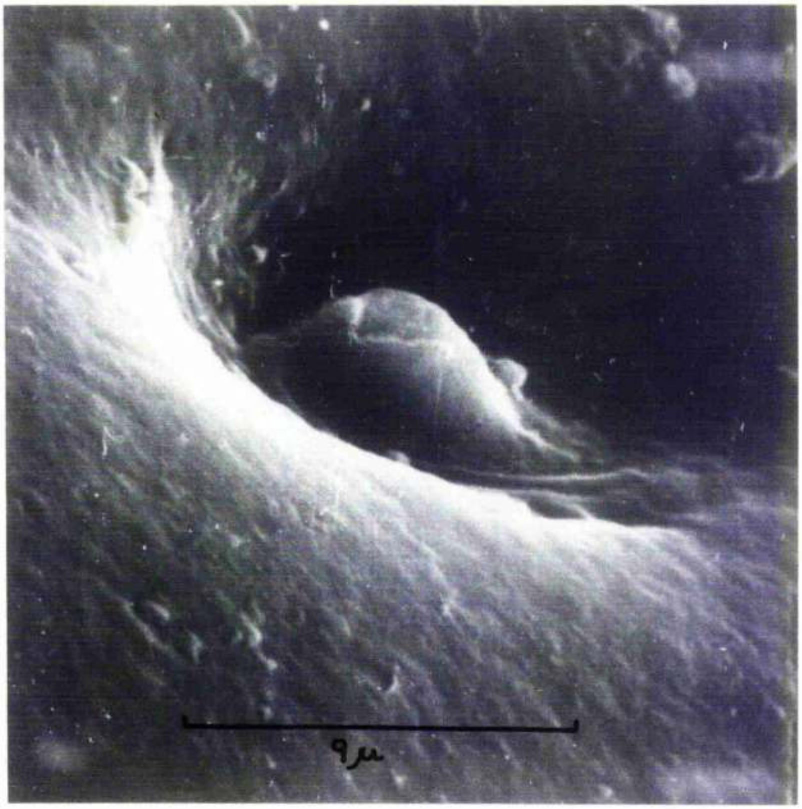


Fig.17. Campaniform Organ.

This is a surface view of the domed cap of a campaniform organ from the dactylopodite segment of a *Carcinus* pereiopod.

Fig. 18. Damaged Campaniform Organ.

Campaniform organs terminate in a delicate domed cap. This is normally absent in light microscope sections and usually "springs off" in preparations dried for scanning electron microscopy as has occurred here.

Fig. 19. Cuticular Gland (Palinurus).

These glands are numerous in the tip region of Crustacean legs and have been confused with campaniform organs (Dennell 1960).

d = gland duct.

multicellular gland of this kind.

It is interesting to recall that Dennell (1960) made the observation that the number of canals (campaniform organs) seemed to be correlated with the degree of tanning and therefore of hardness of the epicuticular pad at the tip of the leg. He postulated that the glands secreted substances involved in the tanning process. The finding that the canals are not gland ducts after all does not, however, alter the plausibility of Dennell's conclusion as to the function of the glands. The fine ducts, the actual ducts of the cuticular glands, are indeed most numerous in Decapoda with a dark-coloured, heavily tanned epicuticle such as Cancer and Palinurus. The reasons why the number of campaniform organs at the leg tip is greatest in genera with a heavily tanned epicuticle will be discussed below under the general topic of campaniform organ distribution.

History repeats itself to the extent that as early as 1938, Pringle reported the confusion which then existed between the openings of cuticular glands and campaniform organs in insects, a confusion which his paper (1938.I) was instrumental in clearing up.

Other Cuticular Sense Organs.

Having considered briefly the campaniform organs we can now proceed to review the other categories of cuticular sense organ which occur on the appendages and carapace.

Mechano-receptor Hairs.

A considerable variety of Crustacean hair sensillae contain only mechanically sensitive units. All these hairs exhibit branching in some degree and the relative development of shaft and branches varies a great deal. In the largest mechanoreceptor hairs the shaft is strongly developed and the branches are no more than a terminal fringe. Hairs of this kind, typically innervated by two or three large neurones,are part of the normal sensory equipment of all Decapoda. Their distribution varies somewhat. For instance, large mechano-receptor hairs tend to occur singly in *Carcinus* and in bunches in *Homarus* (Fig.9). The smaller varieties of mechano-receptor hair normally occur singly in shallow depressions in all genera. The Buschelorgan of *Carcinus* is typical of this group and one is illustrated in Fig.20. These tuft-like, branched hairs were first described by Luther

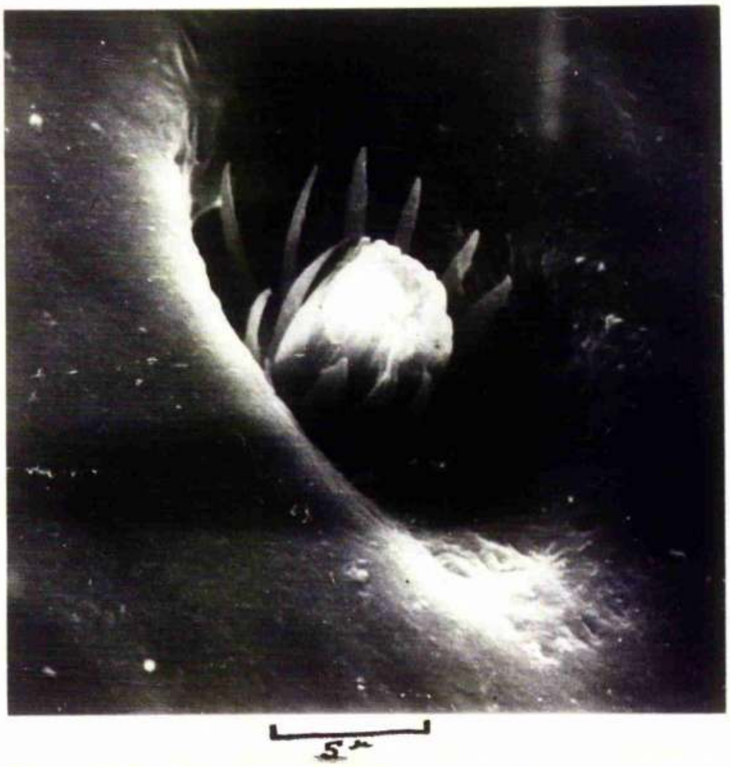


Fig.20. Buschelorgan of *Carcinus maenas*.

Buschelorganen are small branched hairs situated in shallow pits. These organs are present on the carapace, chelae, pereiopods and mouthparts of Decapoda.

in 1930, and like campaniform organs have an almost universal distribution. Laverack (1962) distinguishes between two types in *Homarus* calling those with a well-developed shaft "Hair Peg" organs and those in which the shaft is absent or concealed among the numerous branches "Hair Fan" organs. Intermediate types occur, however, both in *Homarus* and *Carcinus* and it is no longer possible to maintain a distinction on morphological grounds only. Laverack (1962) showed that the longer Hair Peg organs responded to water currents while the Hair Fan organs responded to the transient displacements accompanying changes in pressure. Since both types of stimulus are essentially the same it is debatable whether the distinction between the Hair Peg and Hair Fan types is still a useful concept. In the light of information gained from other species such as *Carcinus* and *Palinurus* it seems that a continuous range of variation in the structure of these small mechano-receptors normally occurs and that while it is easily possible to maintain a distinction between large mechanically sensitive hairs and the small sensillae of the

Buschelorgan type, distinctions within the Buschelorgan category appear increasingly artificial. A certain amount of alteration in the appearance of Hair Peg organs occurs between moults. The central shaft is very liable to wear and is frequently worn level with the branches by the time of the next moult, making a precise classification even more difficult. In all cases a small number of neurones is associated with these small hairs.

Chemoreceptor Hairs.

There is considerable variation in the structure of chemoreceptor hairs in Crustacea and this will be discussed in detail when the chemoreceptor system is considered as a whole.

In the walking legs, with the exception of the chelate pairs of walking legs in *Homarus* , the chemo-receptor hairs are arranged in dense rows. Frequently mechano-receptor hairs are also present in these groups. However, the extensive branching of the chemically sensitive hairs effectively distinguishes them from their mechano-receptor fellows. These mechano-receptor sense organs are normally placed at the periphery of the main chemo-receptor masses. An additional distinguishing feature is the darkly tanned appearance of the mechano-receptor hairs. The branches and also much of the distal shaft region of the chemo-receptor hairs are very lightly tanned and in colour vary from straw yellow to colourless.

The innervation of the branched chemoreceptor hairs of the walking legs strongly resembles that of the aesthetasc hairs on the antennule (Laverack & Ardill 1965). Each hair has a very large number of bipolar neurones associated with it. Possibly as many as 200 enter the base of each hair shaft.

To what extent chemoreceptor hairs exhibit mechanical sensitivity will be considered later. There is no morphological evidence, however, for larger neurones associated with the base of the hair which are specifically mechano-receptor in function. The dendrites of the small bipolar neurones associated with these hairs all appear to extend at least to the base of each branch and quite probably some distance up it. Since these nerve cells form the only apparent innervation there is the distinct possibility that primarily chemo-receptive units are responding to mechanical stimuli.

Interim Summary.

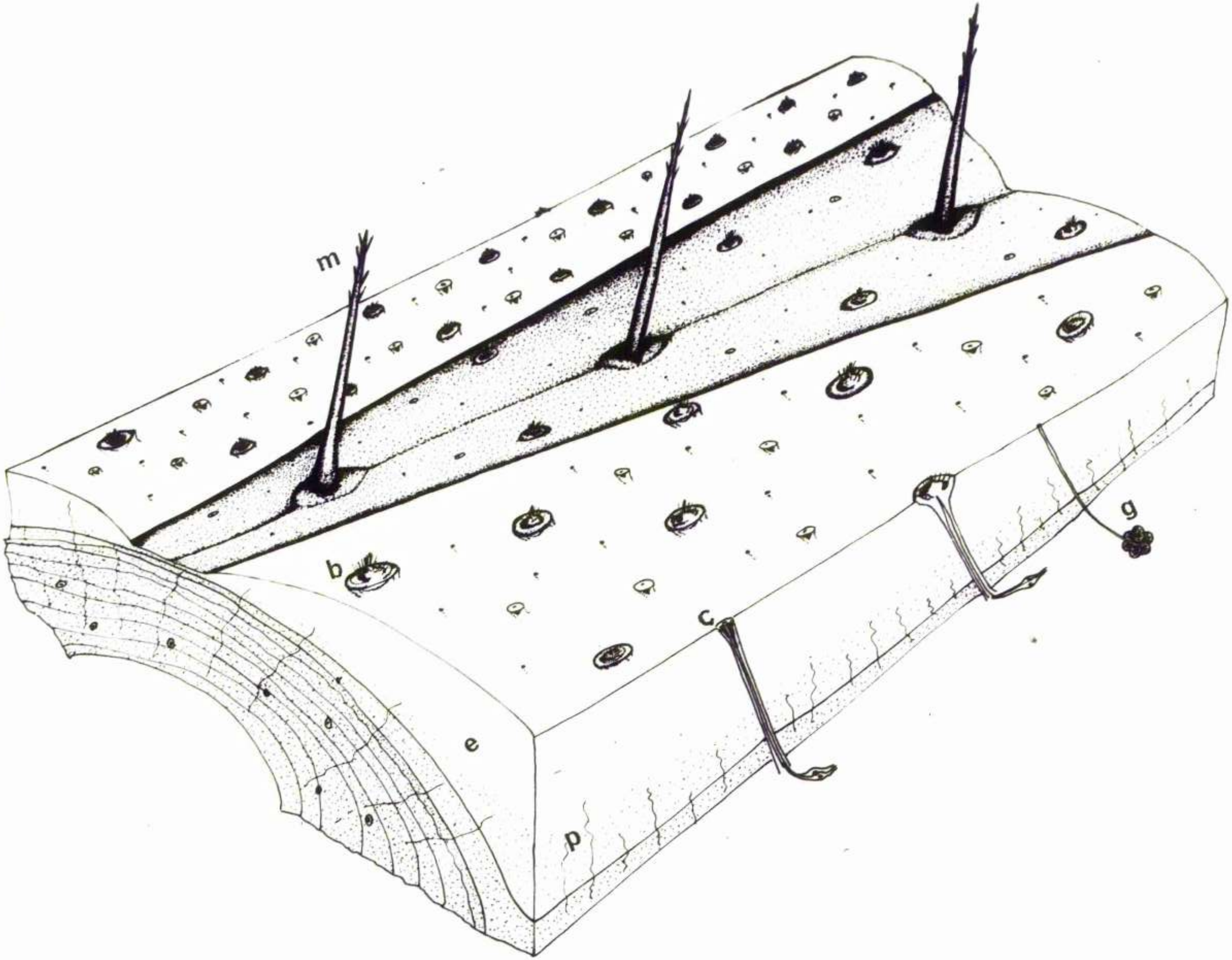
The main findings of the initial part of this study are summarized in Fig. 2I, which resolves the Luther/Dennell argument by distinguishing between funnel canals, gland ducts and pore canals and includes examples of the 3 main kinds of superficial mechano-receptor found in Crustacea.

From here it was possible to examine the physiology and anatomy of the two main organ systems in Crustacea which are least known, namely the campaniform system and the chemo-receptor hair system.

Fig.21. Summary Diagram of *Carcinus* cuticular structures.

This diagram represents a wedge of integument from the tip of the dactylopodite and illustrates the main cuticular specializations discussed in the text and distinguishes clearly between pore canals (processes of hypodermal cells), campaniform organs and cuticular glands.

b) Buschelorgan (mechanoreceptor).
c) Campaniform organ (mechanoreceptor).
e) Epicuticle.
g) Cuticular gland.
m) Mechano-receptor hair.
p) Pore canal.


m
b
g
c
e
p

Physiology of the Crustacean Campaniform Organ.

Sensitivity to Chemical Stimuli.

Since Luther (I930) first made the suggestion that the "funnel canal organ" might represent the morphological basis for the Crustacean contact chemo-receptor system it has been generally assumed that these "funnel canal organs" were a rather specialized and unique kind of chemoreceptor. Direct experimental proof that this is not so depended on two factors.

(I) Finding a preparation which contains an area in which the funnel canal (campaniform) organs are the only sensory structure present.

(2) Applying chemical stimuli accurately to the site of the sense organs under observation and restricting the flow of stimulatory chemicals to that site only.

Campaniform organs are the only sensory structures present in the epicuticular pads at the tips of the pereiopods in *Homarus* and in *Carcinus* the distal part of this same pad is similarly restricted in the number of sensory structures represented.

Both these genera, therefore, provided satisfactory preparations.

Restriction of the region stimulated to the known required area was achieved using a perforated disc of balloon rubber pushed over the tip region of the leg. Stimulation was then applied to the group of campaniform organs distal to the rubber disc.

Experimental Procedure.

(I) Behavioural.

Chemical stimulation of the lower leg normally results in flexion of that limb and in the full reflex the end of the leg is flexed so that it is within reach of the third maxillipeds.

However, when chemical stimuli are restricted, as above, to the tip of the leg it is not possible to elicit the reflex despite the fact that flexion immediately follows when chemical stimulation is applied to regions of the pereiopod distal to the rubber disc.

(2) Electrophysiological.

While the presence of a reflex is an indication that certain organs have been stimulated the absence of that reflex is not, of itself, an indication that stimulation has not taken place. However, the end of the leg can be arranged as an electrophysiological preparation and the nerve fibres associated with the end of the leg can be picked up on a platinum hook electrode. Stimulation being applied as above with the tip exposed in air and separated off from the rest of the dactylopodite by a rubber disc it was impossible to detect any afferent spike activity whatever in the nerve bundles associated with the tip of the leg.

Taken together, these two pieces of evidence effectively disposed of Luther's (1930) concept of a contact chemo-receptor system based upon his "funnel canal (campaniform) organ" and put in question the whole functional significance of these very numerous, small, sense organs.

General Cuticular Sense.

In addition to a sensitivity to the stimulation of specific sense organs (tactile hairs etc.), Crustacea also possess a definite general cuticular tactile sense which does not depend upon stimulation at an actual receptor site but is also present between receptors.

Experimental.

A number of crabs (Carcinus maenas) were blinded by painting the eyes with black paint. Tactile stimulation was then applied by touching the cuticle between receptor sites with a blunt seeker. Frequently the animal would then touch the site of stimulation with the tip of one of the pereiopods, the crab exhibiting considerable accuracy in pin-pointing the stimulated site. This showed that not only is a general cuticular tactile sense present but that it is organized in such a way that it is able to supply precise information about the site of stimulation to the central nervous system of the crab.

Eye Retraction.

Light tactile stimulation of the carapace normally results in eye retraction. Retraction is ipsilateral with a narrow area on either side of the midline of the carapace, the stimulation of which occasionally results in bilateral eye retraction. The reflex can only be elicited by touching the actual cuticle of the carapace and cannot be elicited by stimulating tactile hairs and Buschelorganen.

Receptors of the Cuticular Tactile Sense.

In insects it is well known that the cuticular tactile sense is mediated via campaniform organs which respond to strain in the surrounding cuticle (Pringle I938,I). With the discovery both of a general cuticular sense and of campaniform organs in Crustacea it only remains to be expected that the same situation applied. Electrophysiological evidence that this is so is now available.

Electro-physiology of Campaniform Organs in *Carcinus* and *Homarus*.

The campaniform organs associated with the ends of the legs are exceptional in two respects.

(I) They have a dual innervation.

(2) The bipolar neurones with which they are associated are larger than those of any cuticular sense organ, being equal in size to the bipolar neurones of the elastic strand organs.

The campaniform organs on all other parts of the cuticle have certainly more than 2 functional neurones and these neurones are usually somewhat smaller than the nerve cells associated with mechano-receptor hairs. The majority of campaniform organs belong to this category. Thus, the campaniform organ resembles the Buschelorganen (Hair Peg and Hair Fan organs) in exhibiting a certain degree of dimorphism although it is debatable whether a separate nomenclature should be devised in order to indicate this.

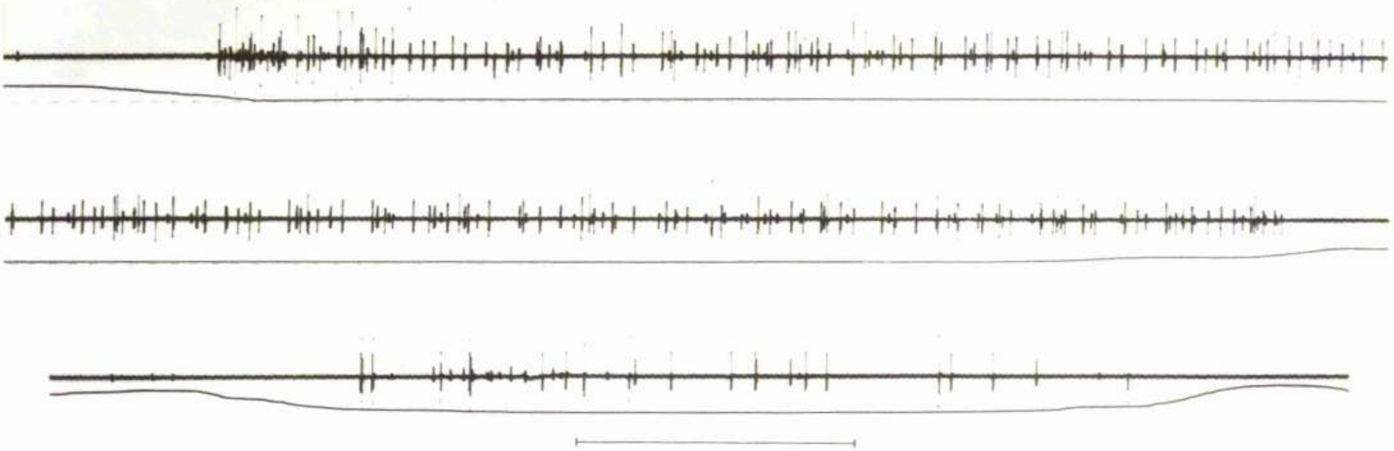
The small, multineuronal campaniform organs are very difficult to isolate and do not provide very satisfactory electrophysiological preparations. Recordings from campaniform organs of this kind form the subject of Fig.22. In both cases straining the cuticle (indicated by the depression of the second beam of the oscilloscope) was the effective stimulus. Fig.22A is a recording from a small number of campaniform organs in a *Homarus* chela where large numbers of small campaniform organs cover both the inner and outer faces of the claw. The campaniform organs are here acting as tonic mechano-receptors and adaptation normally takes fifteen to twenty seconds.

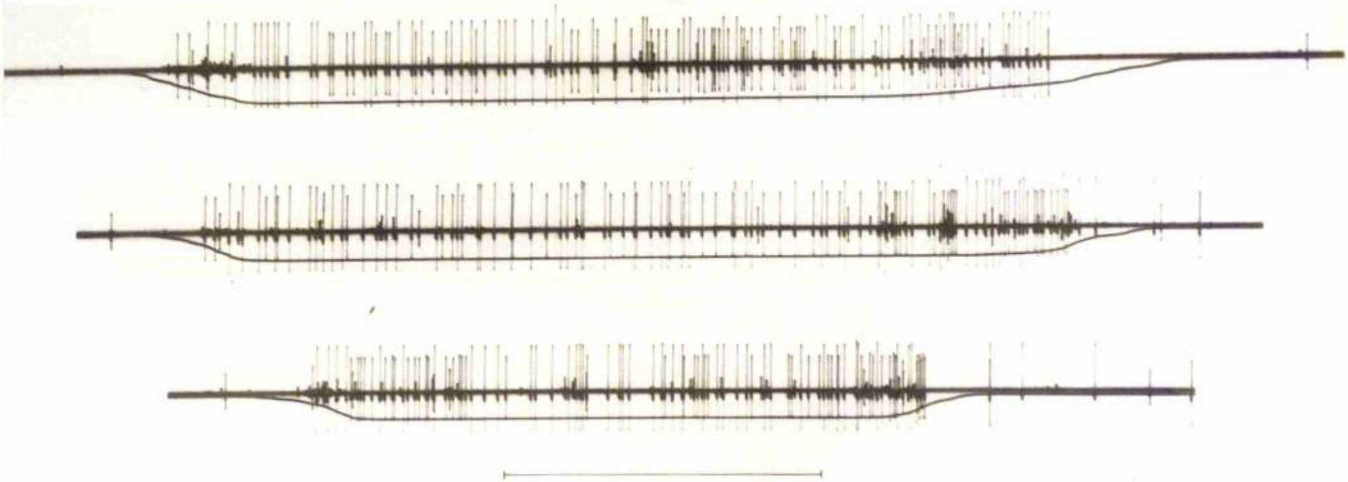
Fig.22B is a recording from a small number of campaniform organs in the propodite segment of a *Carcinus* pereiopod. There is no significant difference in their response to cuticular strain from the *Homarus* chela campaniform organs in the upper record.

Fig.22. Responses from Campaniform Organs in the Chela of *Homarus* (a) and the propodite of *Carcinus* (b).

Basically tonic receptors responding to deflection of the cuticle (lower trace), these organs are innervated by at least three fairly small neurones.

TM = I sec.


a


b

Campaniform Organs in the Tips of Walking Legs.

The relatively large size of the nerves associated with these organs and the relatively small number (in Homarus at least) of campaniform organs present in the tip region of the pereiopod makes electrophysiological isolation of these organs a relatively easy matter.

Straining the cuticle is normally followed by responses in two axons in a completely dissected preparation. This is a consistent and repeatable situation and it is therefore not unreasonable to suppose, especially in view of the histological evidence, that this represents the normal functional innervation of this class of sense organ.

Typical responses to strain are illustrated in Fig.23. In a,b and c,2 neurones, one with a smaller spike potential, are active. The cuticle at the tip of the leg in Homarus is very flexible and capable of considerable deflection. A large deflection of the terminal pad of flexible cuticle always gives rise to a phasic response in the campaniform organs present

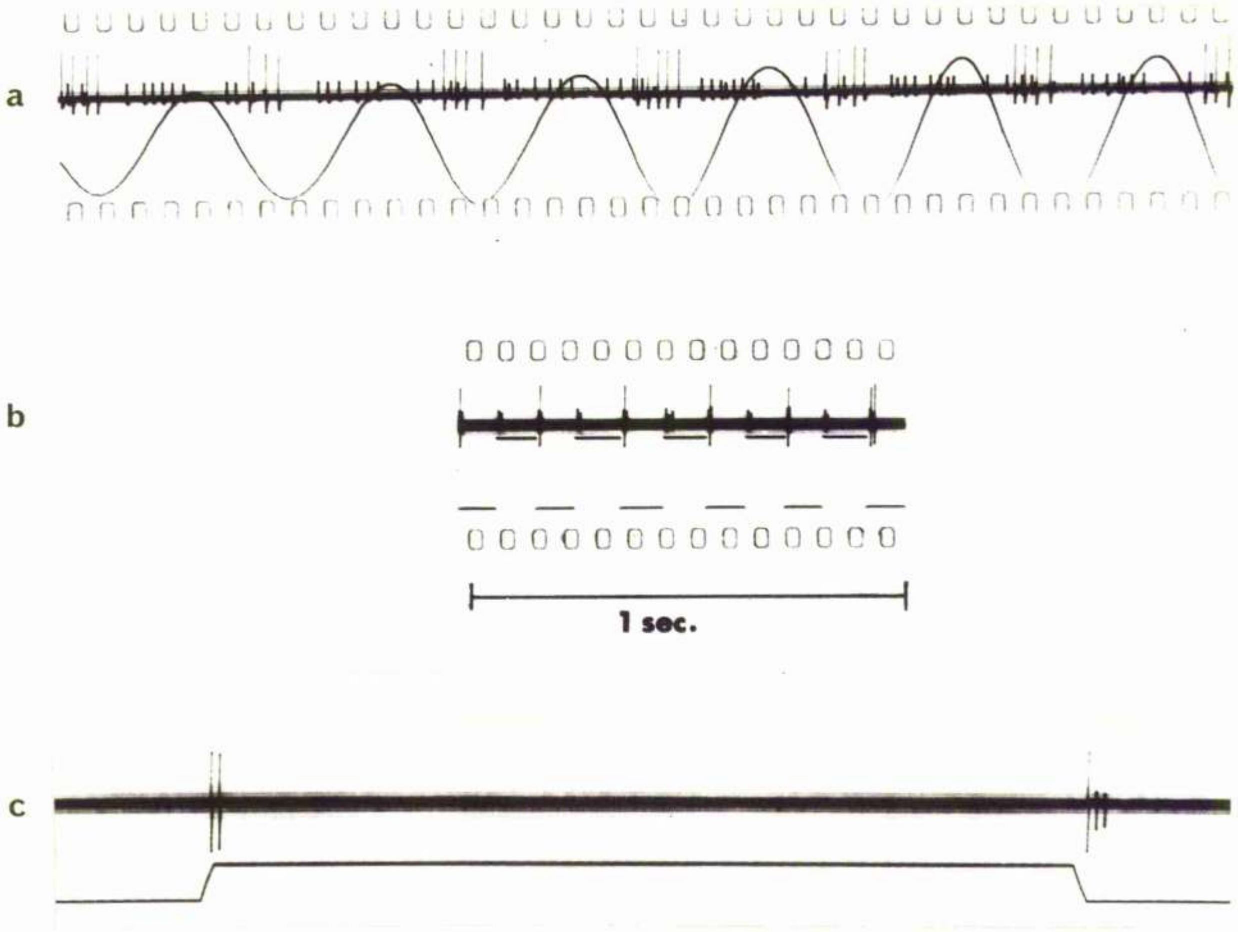


Fig.23. Typical Phasic Responses from Homarus Campaniform Organs from the Leg Tip.

Phasic responses are typical of large deflections of the cuticle.

in this region of the cuticle. It is also, as succeeding illustrations confirm, a consistent observation that one neurone signals the onset of strain while another unit fires as the strain is released. Fig.24 illustrates some typical responses to moderate and light strain in <u>Carcinus</u> and <u>Homarus</u> with the characteristic "on" and "off" units (Fig. 24, a and b). It is possible, however, to stimulate the "on" unit without effecting the stimulation of the "off" fibre as the strain is removed. A very light strain gives rise to a prolonged tonic burst in the "on" unit with no "off" unit activity as the strain is released. This situation is shown in Fig.24, c and d, which is a recording from a <u>Homarus</u> campaniform preparation. The observation that Crustacean campaniform organs respond tonically to small deflections of the cuticle,with or without stimulation of the "off" unit, and phasically to larger deflections, has been a consistent finding of this study and is an important factor to be considered in any theory of the functional anatomy of the cap and peg structure of these sensillae.

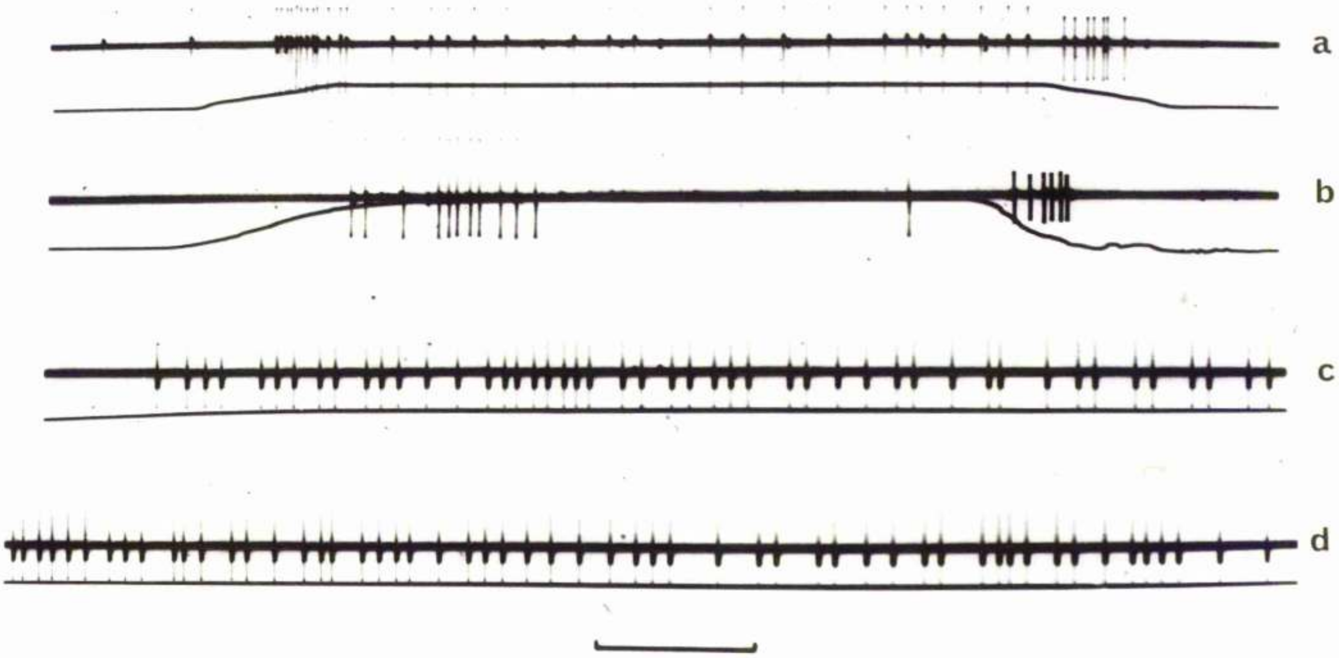


Fig.24. Responses from Campaniform Organs in the Flexible Pad of Decapod Walking Legs.

The campaniform organs of this region are innervated by a pair of large neurones.

a) Response to deflection of the pad in *Carcinus*.

b) Response to large deflection of the pad in *Homarus*.

c)&d) Tonic response to small deflection in *Homarus*.

TM = I sec.

It has also been a consistent finding that campaniform units in *Carcinus* pereiopods show a greater tendency to respond tonically for a given degree of stress than their *Homarus* counterparts. This is almost certainly because the epicuticular pad at the end of the leg is much stiffer in *Carcinus* and therefore much harder to deflect. Viewed in this context the responses from the campaniform organs in the tongue region of the epicuticular pad of *Carcinus* are especially interesting. In *Carcinus*, it will be recalled, the epicuticular pad is not a simple structure but extends proximally up the dactylopodite as a series of tongues which interdigitate with similar tongues of hard, calcified cuticle,(see Fig. 9A). These interdigitating tongues of hard, heavily calcified cuticle greatly stiffen the tongues of the epicuticular pad and consequently it is impossible to deflect this cuticle very far. The responses of the campaniform organs of this region are always tonic. Fig.25 shows the characteristic behaviour of this kind of preparation.

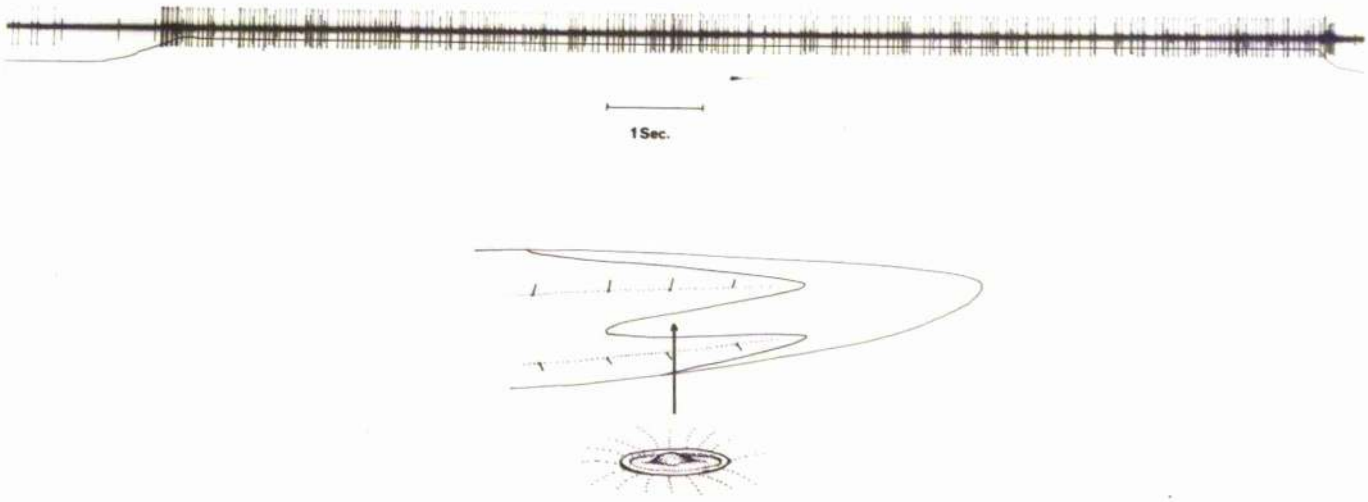


Fig.25. Tonic Response from Campaniform Organ(s) in Tongue Region of *Carcinus* Leg Tip.

The flexible tongues of epicuticle extending proximally from the flexible tip are held rigid by the interdigitating tongues of calcified cuticle. These flexible tongues are therefore limited in the amount of deflection which can be applied to them with a probe. Responses from the large, dually innervated campaniform organs of this tongue region are typically tonic.

Responses to Phasic Stimuli.

Placed, as they are, at the tips of the legs the campaniform organs of this region are ideally sited for vibration reception. In the following experiments probes driven by

(I) A modified oscillograph pen unit (Advance Instruments) and

(2) A modified 5 inch loudspeaker were used to apply vibrational stimuli to leg tip campaniform preparations.

The illustrations of some of the results obtained are all from Homarus material. Similar results were obtained with Carcinus preparations which, however, having a harder cuticle, required slightly more powerful stimulation.

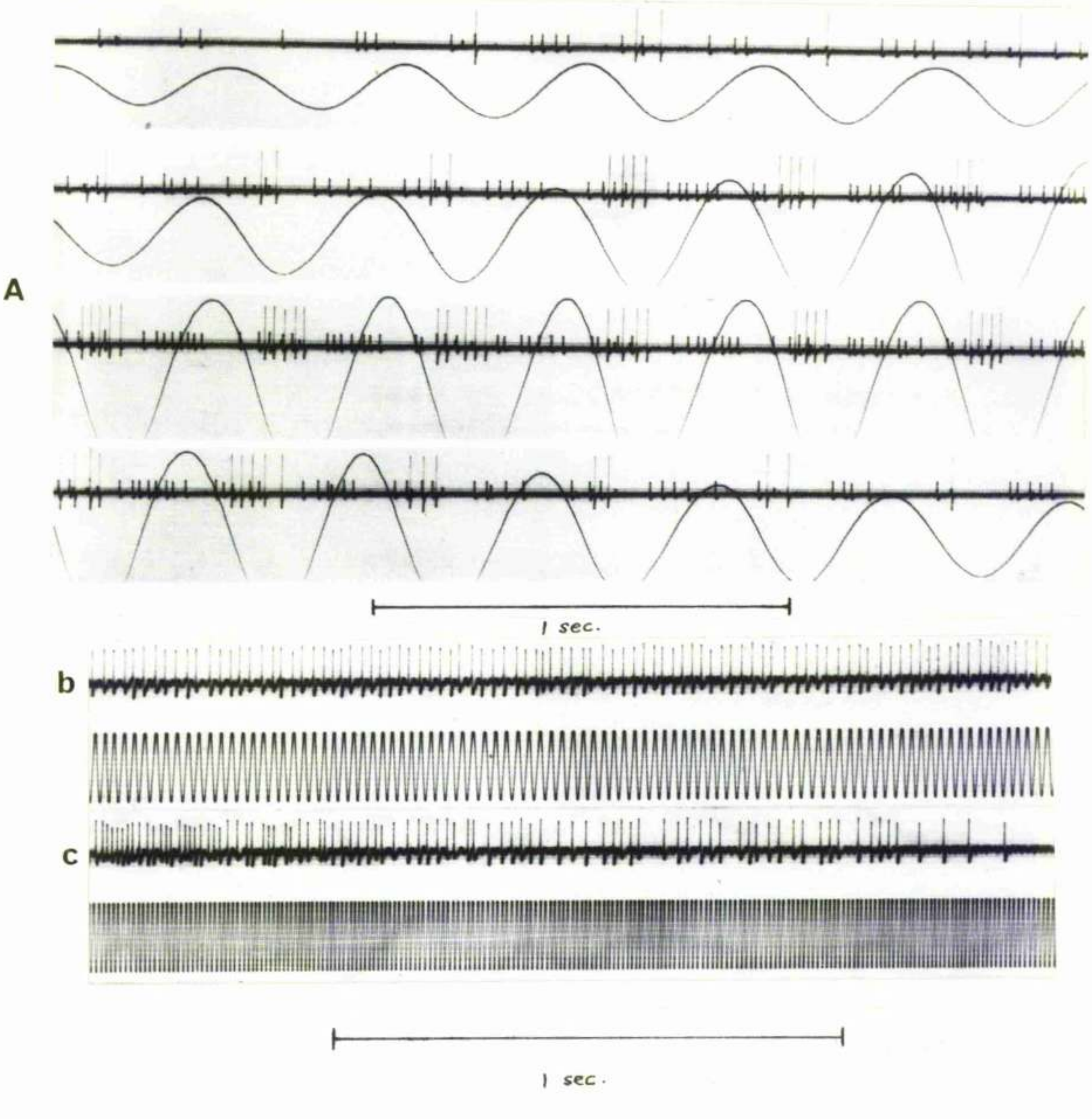
At low frequencies both "on" and "off" units are active. A typical response of this type is illustrated in Fig.26A. The stimulus is monitored on the lower beam of the oscilloscope record. At higher frequencies the "off" unit drops out and the "on" unit fires in a one to one relationship with the stimulus (Fig. 26b) until a frequency of 60-I00 cycles per second

Fig.26.Responses to Vibration in Campaniform Organs of *Homarus* Leg Tip.

A. Typical 2 neurone response to low frequency vibration (in this case of variable amplitude).

b. One to one following by a campaniform organ fibre at moderate frequency.

c. At higher frequency one to one following no longer occurs except briefly at the onset of stimulation.


A
1 sec.
b
c
1 sec.

is reached. At higher frequencies, sometimes after a short synchronous burst, the "on" unit fires once every so many cycles in a fairly irregular manner (Fig.26c).

The dropping out of the "off" unit is a characteristic phenomenon probably explicable on purely mechanical grounds. If one assumes that the epicuticle is a resilient tissue it is reasonable to suppose that a high frequency vibration would produce a smaller degree of deflection for the same power per cycle than a lower frequency. In the same way that a light touch does not always stimulate the "off" unit so the resilience of the cuticle damps the vibration at high frequency and effectively reduces its amplitude to a level high enough to stimulate the "on" unit but not high enough to stimulate the "off" one.

In dually innervated hairs in which one large fibre and one smaller fibre constitute the innervation, one to one following at high frequency occurs in both small and large fibres (Mellon 1963).

These hairs have a very flexible articulation with the carapace so that considerations of mechanical resilience do not apply.

The bipolar neurones of these large hairs are very similar in their characteristics to the pair associated with each large campaniform organ. It thus seems reasonable to suggest a mechanical rather than an electrophysiological explanation for the "dropping out" of the "off" unit of the campaniform organs.

Adaptation to Phasic Stimuli.

Adaptation to vibrational stimuli is, as might be expected, more rapid at higher frequency. At very high frequency, where synchrony of response is soon lost, adaptation is usually within a few seconds of the onset of the response, (Fig. 26c), with a tailing off of the spike frequency until total adaptation.

Adaptation in a preparation responding in a synchronous manner is usually very abrupt with a minimum "tail off" in spike frequency, (Fig.27).

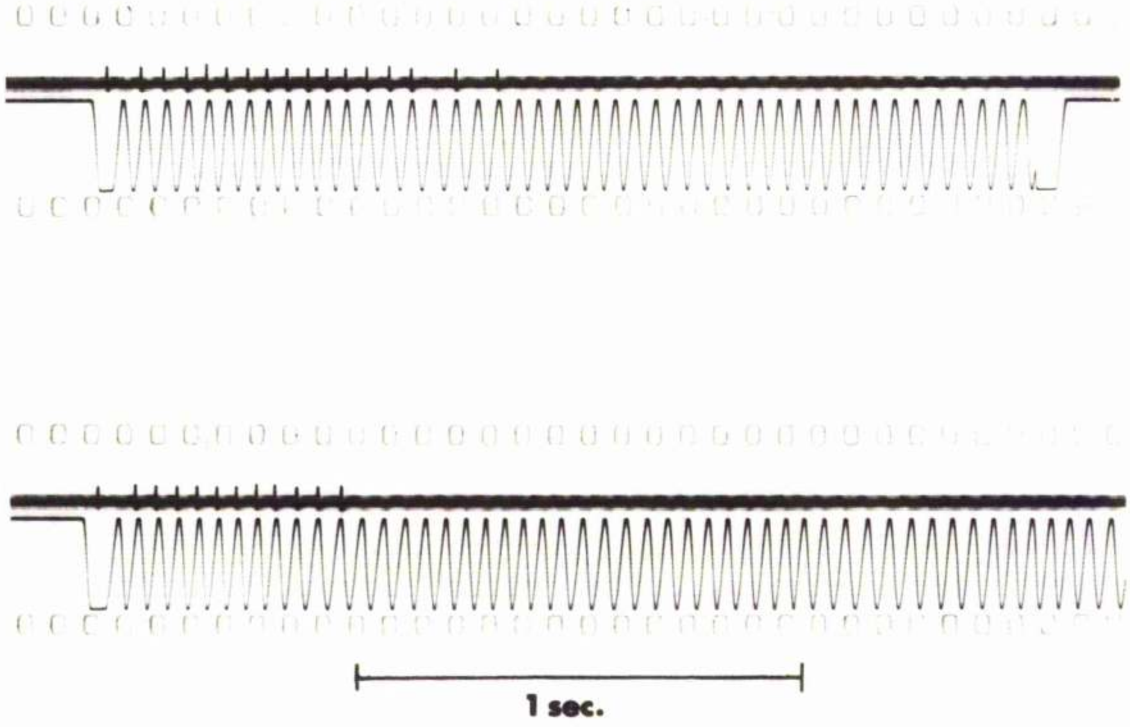


Fig.27. Adaptation to a Phasic Stimulus.

Adaptation to a low frequency phasic stimulus usually takes many seconds. In these consecutive records from a rather fatigued campaniform organ preparation (Homarus) adaptation is occurring quite soon after the onset of stimulation.

A PRELIMINARY THEORY OF THE TRANSDUCTION MECHANISM OF CAMPANIFORM ORGANS.

It has been found (pp. 44-45) that two main kinds of campaniform organ are distinguishable in Decapod Crustacea.

Type (I) Sensillae possess a multiple innervation of relatively small neurones, are widespread in their distribution and have a radially symetrical outer cap, the innervated peg being inserted at the approximate centre of this delicate, outer cap. Type (2) Sensillae are dually innervated by two, large, bipolar neurones. Their distribution is limited to thick pads of epicuticle at the ends of appendages. Unlike the type one sensillae the delicate outer cap is elliptical in the type two variety and the innervated peg is inserted at the distal end of an elliptical cap.

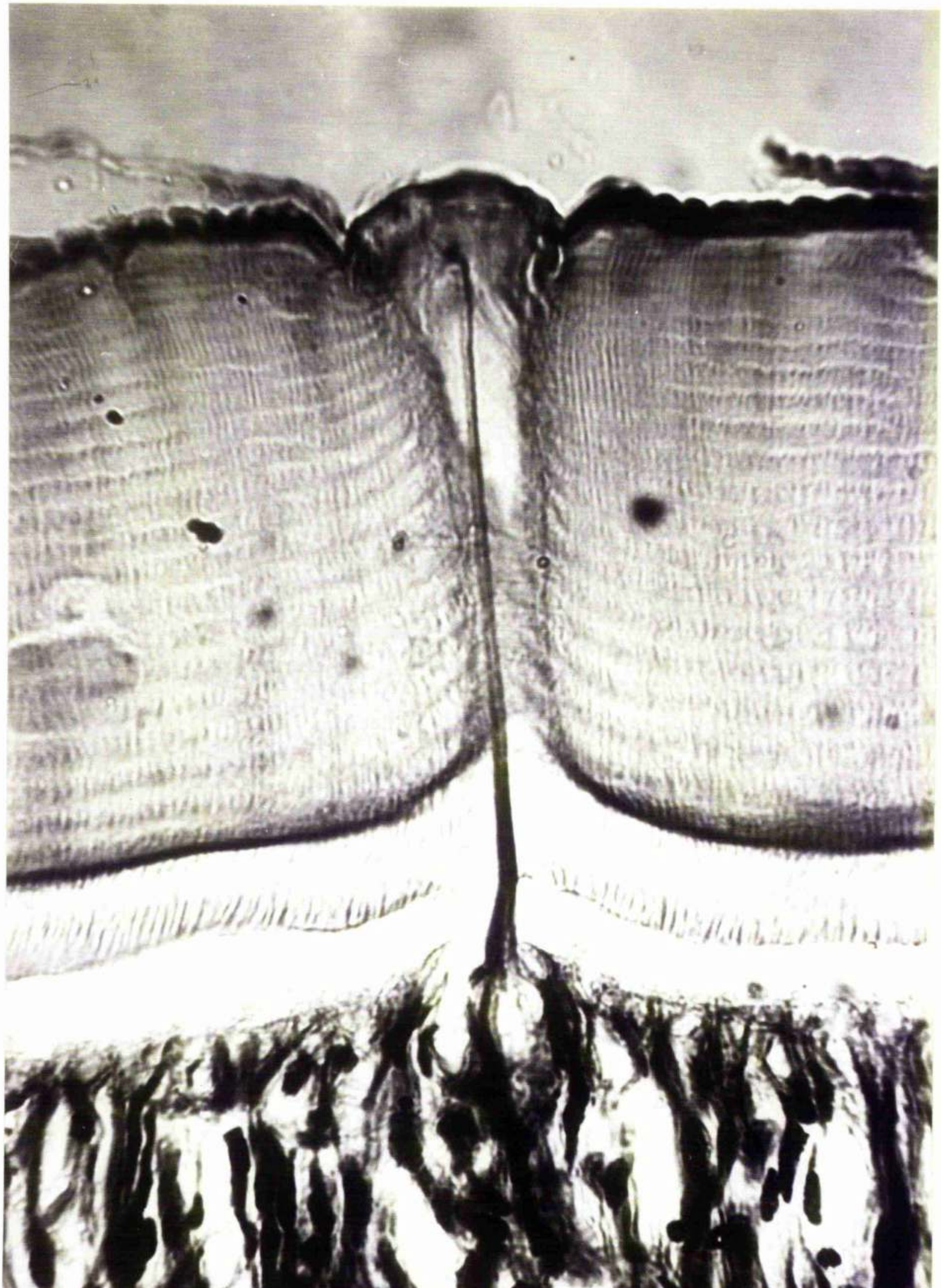
The shape of the tip of the peg at its articulation with the outer cap is the same for both types. At its distal end the central peg is bent at an angle of about 35 degrees from the vertical. This is visible in cleared, whole mounts of Type two receptors and in sections of the Type one receptors (Fig.28).

It is not unreasonable to assume that the outer cap acts as a simple lever which converts small strains in the surrounding cuticle into amplified movements which are communicated to the innervated peg. However, because the tip of this peg is set at an angle to the cap any up and down movement of this component would lead to a side to side movement of the peg, and the dendrites inserted on one side of the peg would be subjected to stretching while those on the other side would be subjected to compression. In the simple situation of the Type two receptors, only two functional dendrites are present and the characteristic "on" - "off" response of these sense organs is compatible with the theoretical scheme expressed above. Whether stretch or compression is the effective stimulus to the dendrite concerned in no way affects this basic theory.

The situation in the Type I receptors is more complex and it is not at all clear whether the dendrites are arranged in "on"- "off" groups or whether they

Fig. Campaniform Organ from Homarus Chela.

In this silver stained preparation by Dr. Margaret Lang (former Gatty histologist) dendrites are seen to enter a central tube ending in a distal, epicuticular cap.



all act together as a single "on" group.

Lack of Directionality in the Responses of Campaniform Organs.

Stimulation of the cuticle around the full 360 degrees surrounding a campaniform organ always leads to the same all or none response of the organ and there is no evidence for the directional type of response typical of large tactile hairs. However, elliptical, Type two organs are more sensitive along the long axis of the elliptical cap than the short one. This is to be expected as a consequence of the assumption that the cap acts as a simple lever amplifier. The greatest sensitivity would, therefore, be expected where the lever is longest, i.e. along the long axis of the ellipse.

The angled structure of the peg means that the angled tip can move in one plane only no matter how and from what point the cap is moved. A tactile hair can move in a number of different planes about 360 degrees and the group or pair of neurones associated with it are consequently free to respond in a typical directional manner. The all or none "on" - "off" response of the campaniform organ is a necessary consequence of the bent peg structure which restricts the innervated region to movements in one plane only.

THE SPATIAL ORGANIZATION OF THE CAMPANIFORM ORGAN SYSTEM.

Integration of Campaniform Input.

Because the response of a campaniform organ is not directional, it follows that a group of campaniform organs cannot supply the kind of information which would enable the central nervous system to triangulate the exact position on the cuticle which is under strain. The site of stimulation can only be determined by integrating the intensity of response from the individual campaniform organs in a stimulated group. This is inferior to triangulation as a means of fixing the site of stimulation and a far higher density of sense organs would be required to give the same tactile acuity.

These considerations are helpful in understanding the observed distribution of campaniform organs over the surface of the body.

Appendages and parts of appendages which are used to manipulate external objects, such as chelae, mouth parts etc., are covered by a very dense population of campaniform organs and consequently possess high tactile acuity. In the case of pereiopods the density of campaniform organs is highest at the tip and dactylopodite and decreases to a much lower level in the upper segments (see Fig.29).

Economy of Sense Organs.

There are almost no places on the surface of a Crab or Lobster which are completely insensitive to touch, despite the fact that in places with low tactile acuity, such as the upper segments of the walking legs and the back of the carapace, campaniform organs occur at a very low density level. In these low density situations the campaniform organs are always placed in close proximity to tactile hairs or Buschelorganen. Indeed on the back of the carapace and on the meropodite joint of the pereiopods practically every Buschelorgan is associated with one or more campaniform organs (Fig.30). Pringle (1938,2.), when discussing the distribution of campaniform organs in insects, described the tendency for campaniform organs to be associated with the bases of large,

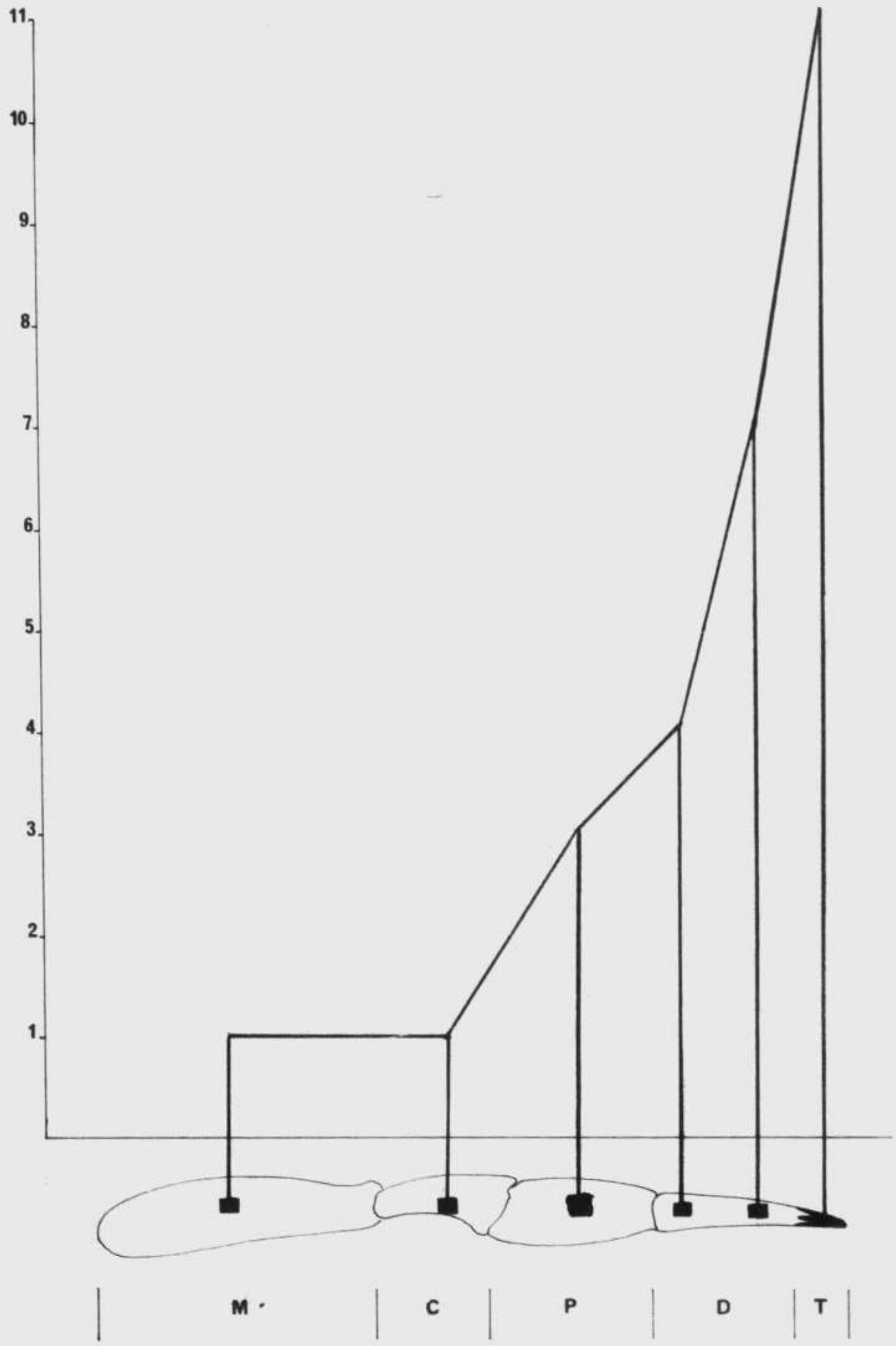
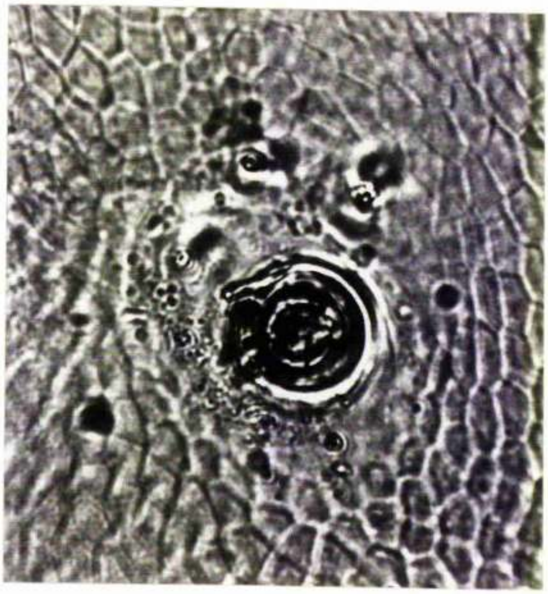


Fig.29. Distribution of Campaniform Organs in a <u>Carcinus</u> Pereiopod

Five standard areas (220x220 micra squares) were examined per leg segment (mero M, carpo C, pro P, dactylopodite and tip) and the campaniform organs counted. Average values per segment were then plotted on the "Y" axis of the graph against position on the leg ("X" axis).

Fig.3a Cleared Cuticle Preparations to show Pairing Phenomena.

These three photographs illustrate the way in which campaniform organs (small structures) and Buschelorganen (large circles) associate in groups. The possible reason for this is discussed in the text. All three specimens are from the meropodite region of a *Carcinus* walking leg.



tactile hairs and suggests that the campaniform organs respond to strains set up in the hair base cuticle when the hair is moved. It is possible that the same applies to the campaniform organs at the base of large, tactile hairs in <u>Carcinus</u> and <u>Homarus</u> but there is no physiological evidence for this. However, in the case of the campaniform organs at the base of the Buschelorganen there is evidence which clearly implies that the campaniform organs are not stimulated when the Buschelorganen are deflected. Streams of water and light brushing readily stimulate the Buschelorganen and their activity can readily be recorded. However, the larger units associated with the campaniform organs are only stimulated when the cuticle is actually touched. Scott (personal communication) describes a very elegant behavioural confirmation of this. When the carapace is touched eye retraction normally follows. However, stimuli which are known to be effective for Buschelorganen, streams of water etc., are quite ineffective in initiating eye retraction.

An alternative theory to explain the pairing between the Buschelorganen and the campaniform sensillae is based upon a consideration of the distribution of stress in the cuticle. It is apparent from the experiments with models which constitute Fig.31 that holes tend to act as stress foci and that between a pair of holes in a stressed lamina a very strong stress line occurs provided the two holes are close enough together. It is here proposed that the large hole present below a Buschelorgan acts as a stress focus and that the campaniform organs are placed in the vicinity of these foci for this reason. By this means large areas of cuticle are rendered sensitive to touch by a minimum number of sense organs. This is a very efficient arrangement for situations where a high degree of tactile acuity is not required. It can also be added that an additional focusing effect takes place as a consequence of the diminution in the thickness of the cuticle due to the fact that

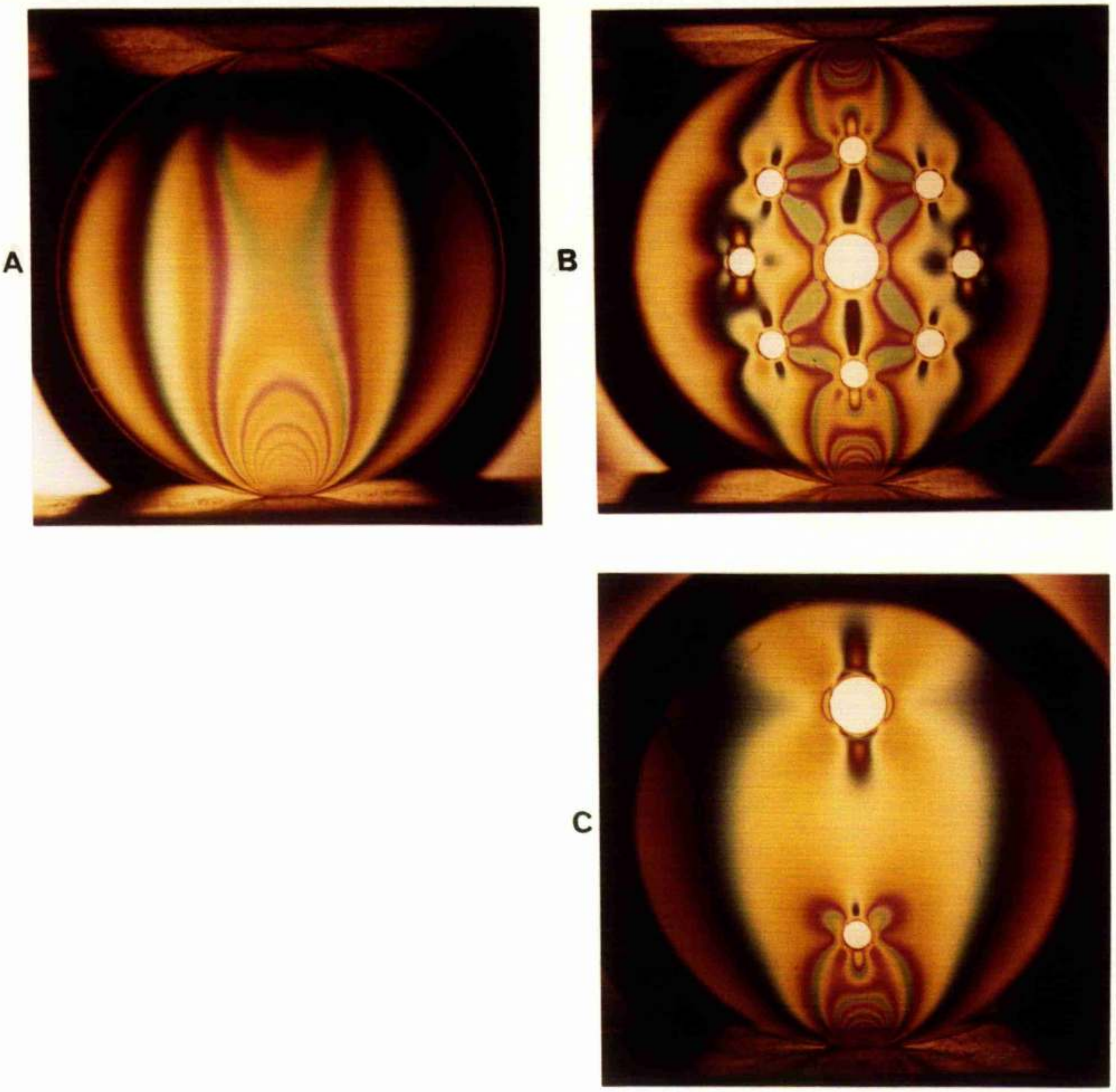


Fig.31. Fringe Patterns in Stressed Plastic.

A. Stress applied to imperforate lamina.

B. Stress in a perforated lamina showing interference between pairs of holes which concentrate the highest stress (green).

C. Stress in a perforated lamina in which the inter-hole distance is too great for stress concentration between the two holes.

Buschelorganen are always placed at the bottom of a definite pit.

Distribution of Type 2 Sensillae.

As mentioned above these large, dually innervated sense organs are found in the thick pads of epicuticle at the ends of the appendages. However, in the Spiny Lobsters *Palinurus* and *Panulirus* the carapace (Fig.32) and the bases of most of the appendages are armed with large spines. In structure these spines strongly resemble the ends of the legs , being large, thick pads of epicuticle. However, this resemblance goes no further than this. These spines do not contain a complex of Type 2 campaniform sensillae,and Hair Peg organs are usually the only sensory structure represented (Fig.33).

The complexes of Type two sensillae in the ends of the appendages are thus unique and it is not

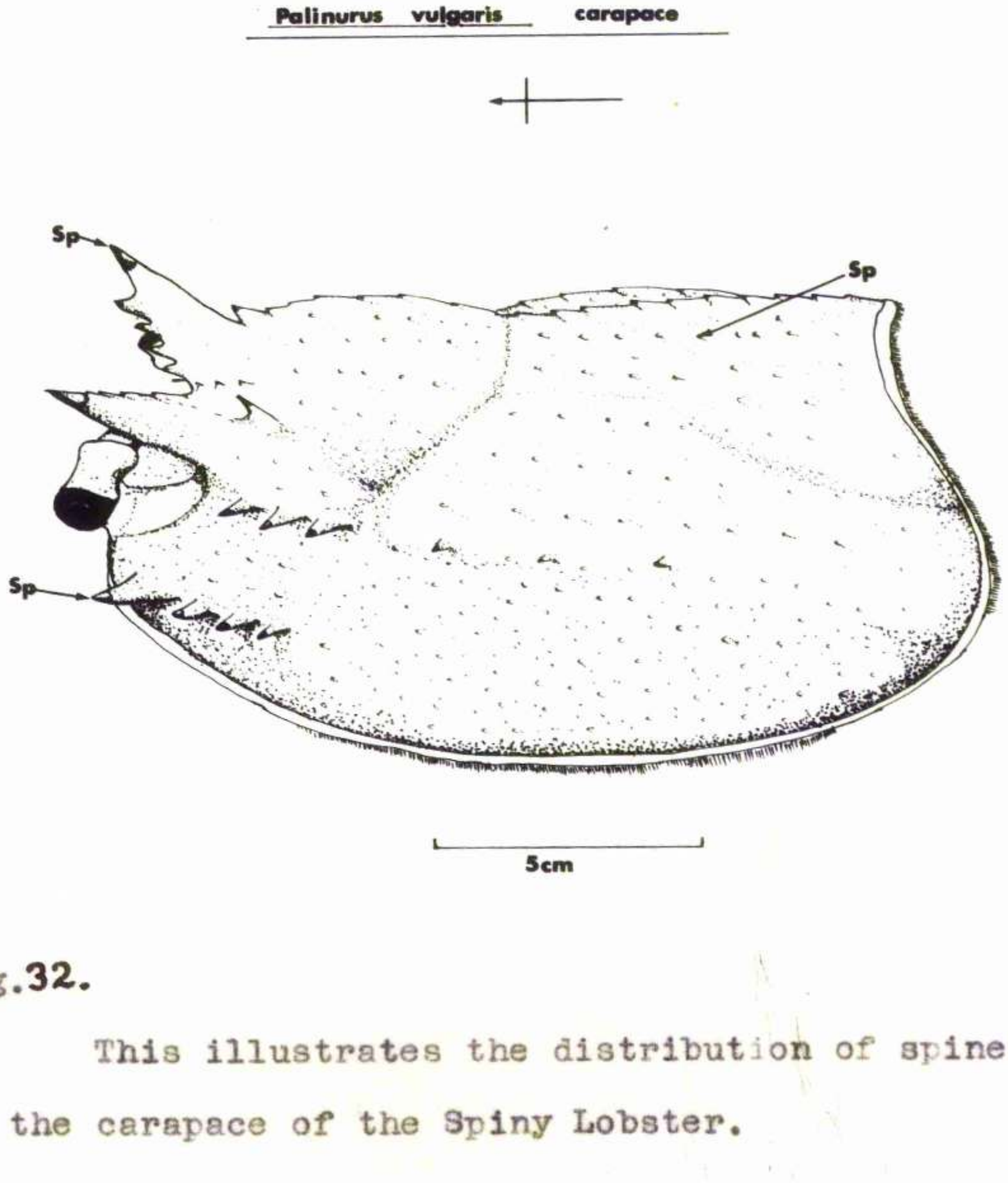


Fig.32.

This illustrates the distribution of spines on the carapace of the Spiny Lobster.

Hair-peg organs in P. vulgaris carapace spine

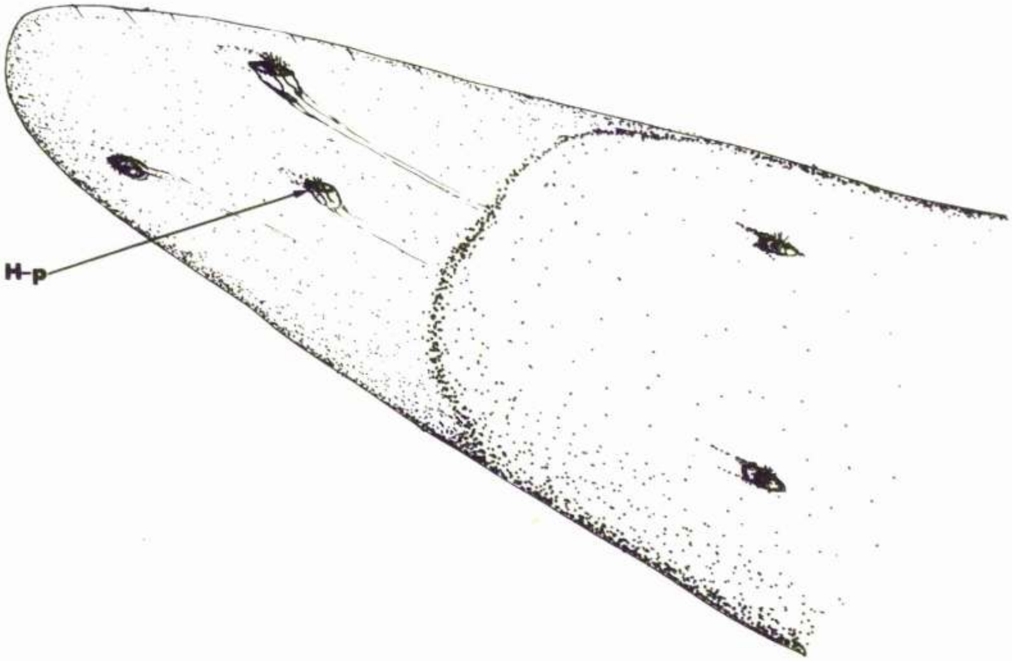


Fig.33.

As at the tip of leg the spines have a greatly thickened pad of epicuticle. However, there is no campaniform organ complex comparable with the leg tip and usually only Hair Peg organs (H-p) are present.

unreasonable to consider these aggregations as composite cuticular sense organs. In walking, these pads are the only direct contact between the animal and the ground (Fig34A).

They would be stimulated whenever the leg involved was in contact with the ground and would also act as receptors for vibration. The high tactile acuity of these pads would also be important in the precise manipulation of external onjects.

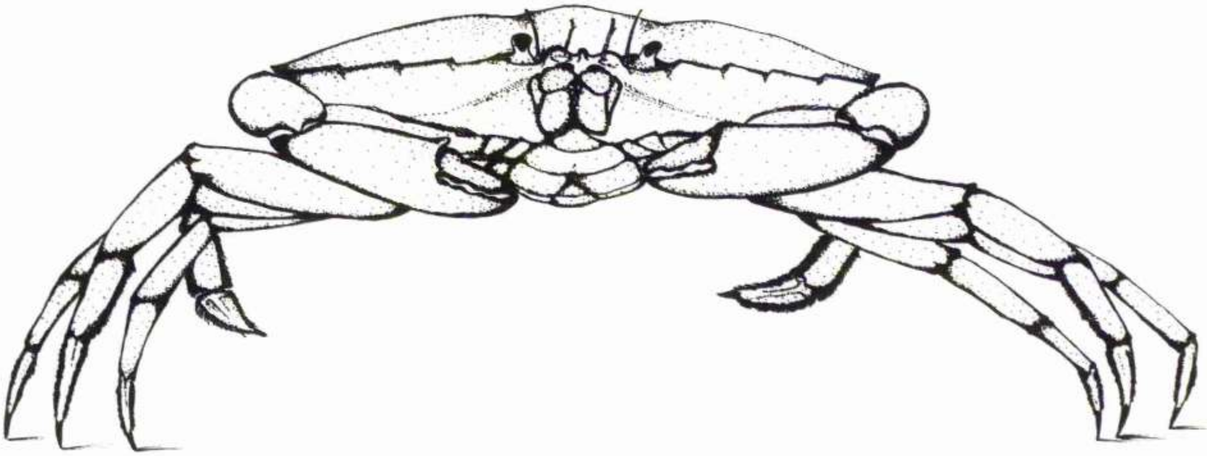
To what extent the input from these pads plays any part in the co-ordination of walking movements remains undetermined. Denervation of the tip, by cutting a hole in the dactylopodite and then severing the afferent nerve fibres, does not affect the walking sequence, neither does shielding the leg pads from stimulation by pushing soft rubber tubing over the ends of the legs. However, it would be a strange situation indeed if this campaniform complex gave rise to no reflex motor activity and an intracellular study of leg muscle fibres could be expected to give

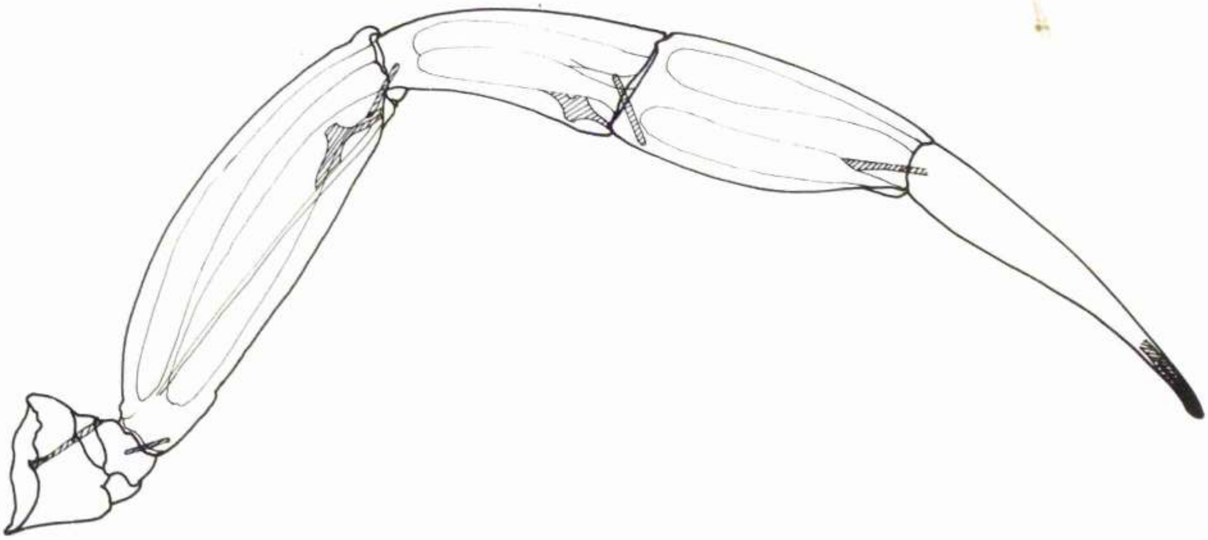
Fig.34. A.Normal Stance of Alert *Carcinus*.

In normal walking, the tip of the leg only is in contact with the sea floor so that the large campaniform organs present here are the only *direct* sensory contact between the crab and the substratum.

Fig.34- B.Proprioceptors of the Leg.

This is a modification of Whitear's (I96) diagram to show the location of leg proprioceptors in *Carcinus*. The large campaniform organs of the leg tip are here tentatively added to this series.





positive evidence of motor activity as a consequence of the stimulation of the leg pad complex.

In Fig.34B the leg pad complex is drawn as part of the series of chordotonal organs which monitor the movements of each leg joint. In Fig.34B the terminal pad is interpreted as the most distal of the chordotonal series, thepad monitoring movements between the ultimate leg "joint", namely that between the dactylopodite and the substratum. As a complex of dually innervated units embedded in a piece of elastic cuticle the leg tip is in many ways analogous to a true elastic strand organ. Some support to this concept is given by the existence of certain other aggregations of dually innervated campaniform structures, the function of which remains obscure, but which appear in some respects to represent a link between true internal elastic strand organs and purely cuticular sensory structures.

Other Aggregations of Campaniform -like Structures.

In addition to the two kinds of true campaniform organ described in this present study a third category of campaniform-like organ has been reported. These tend to occur in small groups at the joints of appendages and even at each joint of both the antenna and the antennule including the annuli of the flagella.

Wiersma (1959) describes "slit sensillae" occuring in groups just distal to the ischio-meropodite, mero-carpodite and carpo-propodite joints of *Homarus* and *Palinurus*. The nerve cells for these "slit sensillae" are associated with true elastic strand organs and are sensitive to touch. Whether these sensillae are part of the normal proprioceptor components of each elastic strand organ is at present under investigation (Clarac, personal communication). Wiersma suggests that the "slit sensillae" may represent an intermediate stage in the evolution of true, internal proprioceptors. This view finds support in the studies of Howse

(1965) on the subgenual organ of an insect *Zootermopsis angusticollis* where campaniform-like organs also co-exist with true scolopidia and Howse recalls Berlese (1909) in suggesting that scolopidia might have evolved from campaniform organs.

Laverack (1964) describes the presence of proprioceptive nerve cells at each interannular articulation of the flagellum of *Panulirus argus*. Although the distal endings of the dendrites were not seen it is extremely likely that they are inserted in the groups of campaniform -like structures which occur at each annulus, not only in *Panulirus* but also in *Homarus* (Fig.35A). A precisely similar situation occurs in insects (Fig.35B). Recently Taylor (1967) has described campaniform-like structures from the basal joints of the antenna of a hermit crab (*Petrochirus californiensis*) which act in a fully proprioceptive role.

We can begin to see, I think, that the complex of campaniform organs at the end of each leg can quite legitimately be regarded as a chordotonal organ.

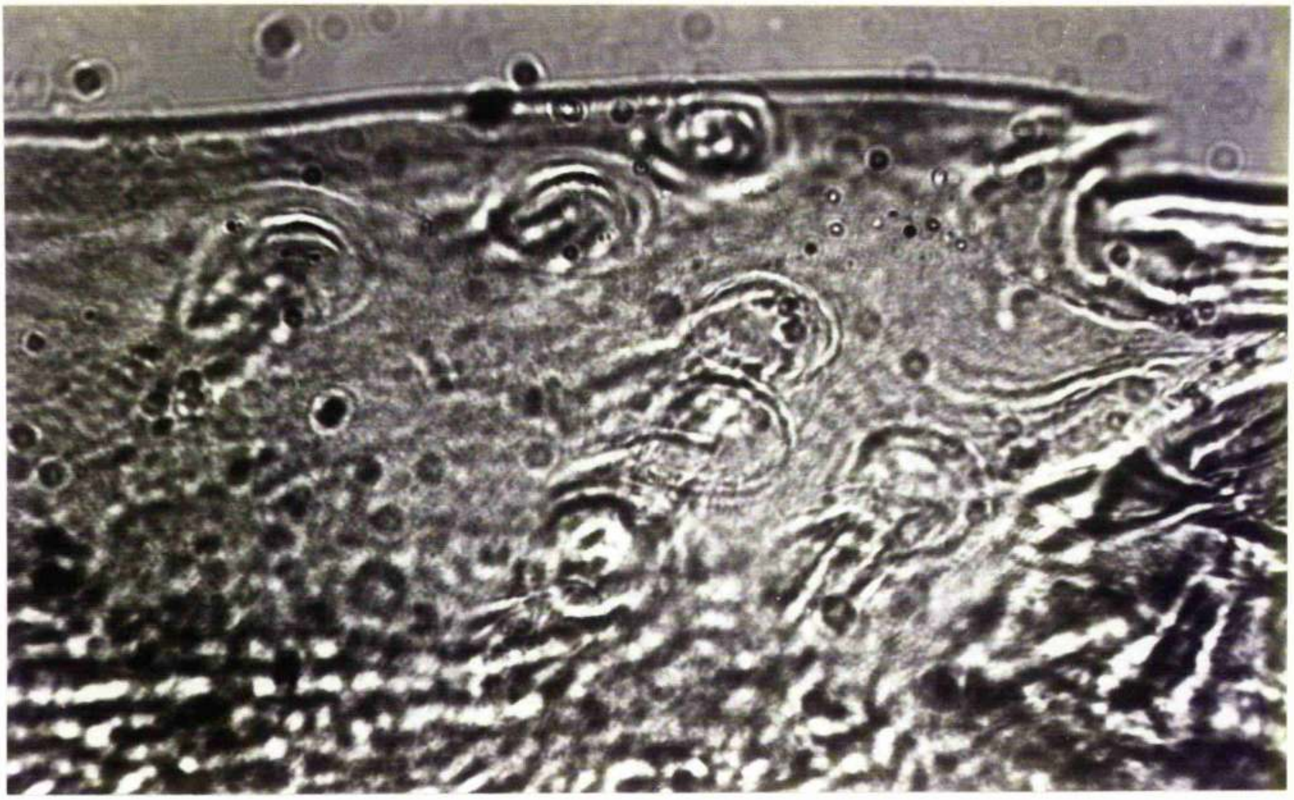
Fig.35. Aggregation of Campaniform Organs.

The Crustacean campaniform organ resembles its Insect counterpart in its distribution.

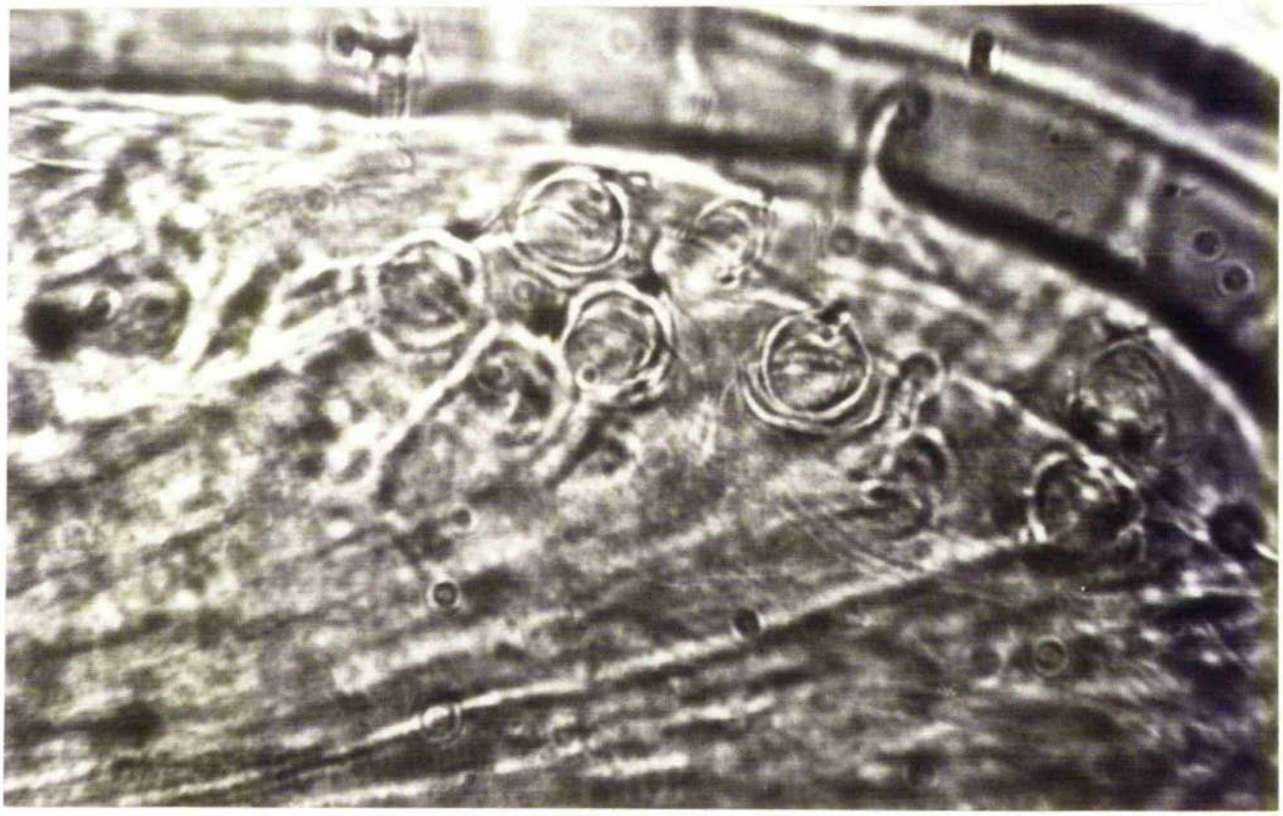
A) Group of campaniform organs from antenna of Homarus.

B) Group of campaniform organs from antenna of Dytiscus (Coleoptera).

Serially repeated groups of campaniform organs are typical of the Insect antenna.



A



B

Similar complexes of dually innervated receptors occur in insects (Fig.36) (Zacharuk, 1962) and these are also dually innervated in contrast to most insect campaniform organs which have a single functional neurone although as many as three may be present during the morphogenesis of the organ (Howse, personal communication).

Dual Innervation in Campaniform Organs & Chordotonal Organs.

In the Type 2 campaniform organs of Crustacea the functional significance of the two neurones is clear enough in the typical "on-off" response of these organs. The reason for the retention of this arrangement was by no means so clear in the case of elastic strand organs. However, recently Hartman and Boetigger (1967) have recorded from single pairs of neurones in the propus/dactylus organ of *Cancer irroratus* and have demonstrated that both units of the pair discharge together and that differences between position (tonic)

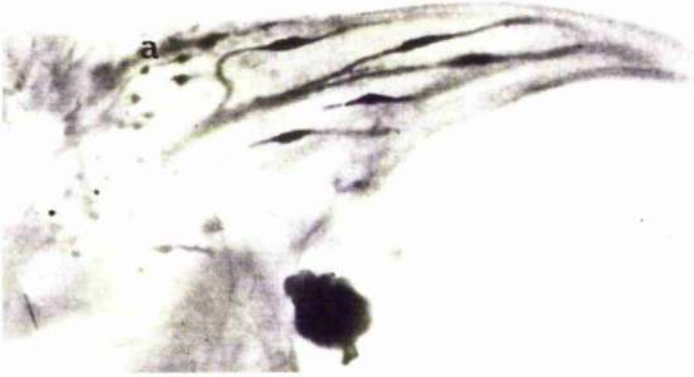


Fig .36. Dually Innervated Campaniform Organs in an Insect.

This is an illustrationfrom Zacharuk's paper (1962) showing dually innervated sense organs from the mandibles of an Elator beetle larva. The better known singly innervated campaniform organs are present at "a".

and movement (phasic) receptors is based not upon distinctions within pairs but upon the point at which a particular pair is inserted in the elastic strand. In other words, one member of the pair is effectively redundant.

THE CHEMORECEPTOR HAIR SYSTEM.

Introductory.

With the finding that Luther's "funnel canal organ" was not a contact chemo-receptor the whole problem of contact chemo-reception in Crustacea was put in question. Accordingly, the pereiopods, chelae and mouthparts, the three main situations where a contact chemoreceptor system is said to exist, were subjected to an anatomical and electrophysiological examination. Three general questions were considered.

(I) What are the receptors involved?

(2) Is their structure constant for all situations in which contact chemo-reception can be shown to exist?

(3) Is contact chemo-reception a meaningful concept for an aquatic animal anyway?

Effectively the study consisted of an anatomical investigation of structures, shown to be chemoreceptors by behavioural and electrophysiological means. In all cases chemical stimulation was applied to structures exposed in air and rubber discs, petroleum jelly and insect wax (a mixture of rosin and beeswax) were used to restrict the flow of stimulatory chemicals to small, known areas. In the case of electrophysiological experiments the innervation to these small, known areas was dissected out in preparation for extra-cellular recording using platinum hook electrodes.

Chemo-receptors of the Pereiopods.

Chemical stimulation of the pereiopods normally results in the flexion of the stimulated appendage (Wiersma I96I). However, this reflex can only be elicited when the stimulatory solution flows over the dense hair rows which occur on the dactylopodite and propodite of *Homarus* and *Carcinus*.Two kinds of hair occur in these rows, (I) heavily tanned, large hairs with sparse, terminal branching and (2) thinner liberally branched hairs with a lightly tanned proximal shaft but untanned branched distal shaft. Electrophysiological experiments indicated that a response to chemicals depended upon the presence of the latter kind of hair and that the larger heavily tanned hairs responded only to tactile stimulation. These latter hairs occur singly in Carcinus and in large, dense bunches in Homarus.

Typical responses to chemical stimulation of the hair rows in Carcinus and Homarus are shown in Fig. 37, and Fig. 38 is a series of recordings of responses to tactile stimulation of the heavily tanned mechanoreceptor hairs of Homarus. The movements of the stimulating probe are monitored on the second beam of the oscilloscope.

There was no evidence, either behavioural or electrophysiological for the existence, on the surface of the pereiopods, of any chemo-sensory structure other than the branched hairs described above, and thus it may reasonably be assumed that these hairs are the only chemosensory structure present on the walking legs.

Hair Plate Organs.

The first two pairs of pereiopods in Homarus are chelate, i.e. the propodite bears a

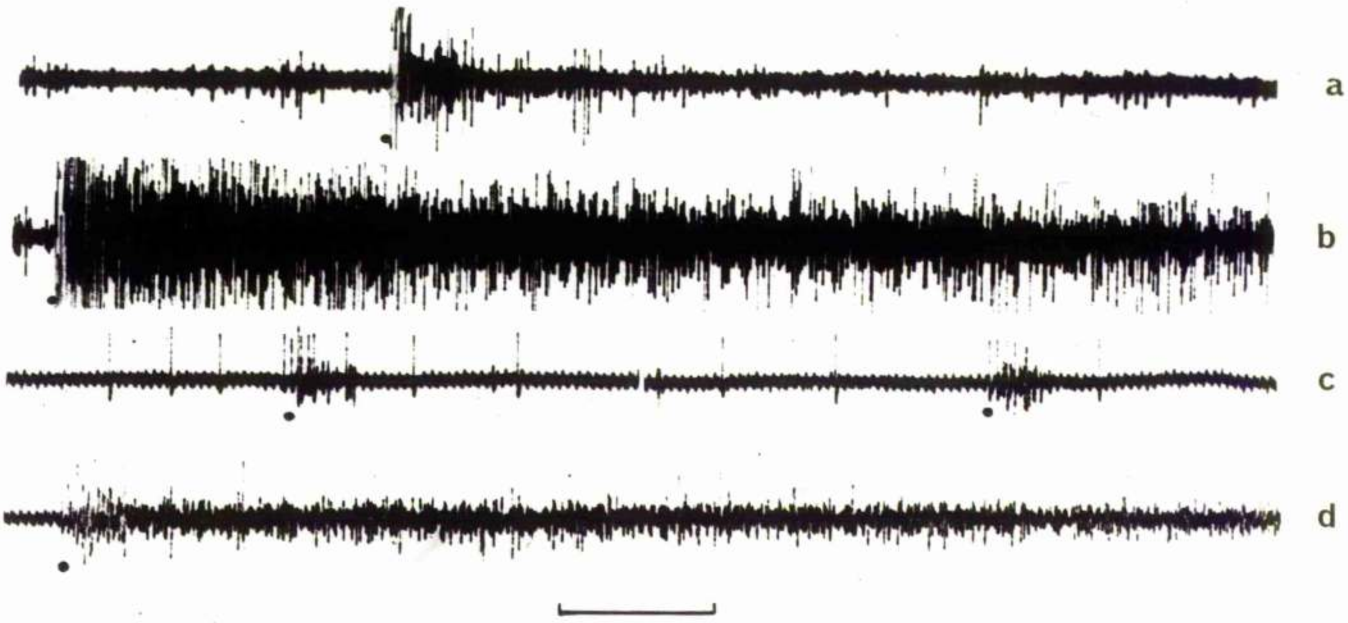


Fig.37. Chemoreceptor Activity from Decapod Walking Leg.

Response to

a) I drop of sea water applied to Carcinus hair row,

b) I drop of filtered Mytilus extract applied to same preparation,

c) 2 drops of sea water applied to Homarus hair row,

d) I drop of filtered Mytilus extract applied to same preparation.

In both cases the preparation consisted of a section of a chemoreceptor hair row from the third pair of walking legs.

Tm = I sec.

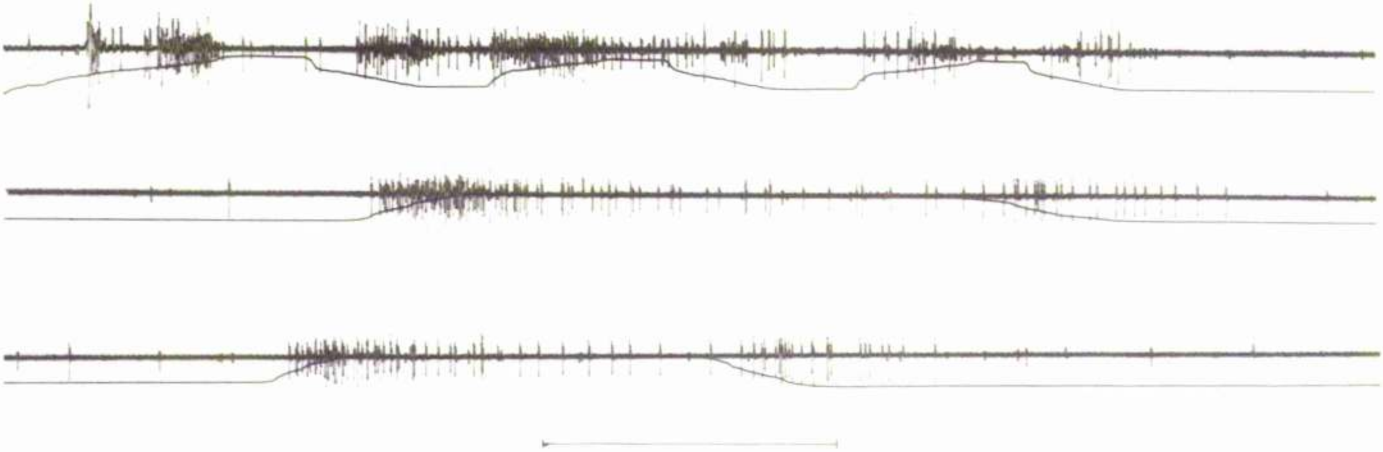


Fig.38. Mechanically Evoked Response from Hair Bunches from a *Homarus* Walking Leg.

These hair bunches are chemically insensitive but respond to mechanical deflection.

Lower trace records movement of deflecting probe.

TM = I sec.

prolongation which is opposed to the dactylopodite. The midline of both inner surfaces is marked by a sharp line which, when magnified, is seen to consist of a row of short, conical hairs (Fig.39). The only other large hair structures present are the dense bunches of mechano-receptor hairs which are present on all the walking legs.

The claw closing reflex can be elicited either by stroking the line of conical hair-plates with a probe or by chemical stimulation of the row with drops of stimulatory chemicals dissolved in sea-water. Stimulation with sea-water alone does not elicit the reflex neither does mechanical stimulation of the bunches of mechano-receptor hairs.

Only one hair type occurs in the central hair row, the conical hair plate organs. Therefore, either each hair plate contains mechanically sensitive units and chemosensory units or every unit of a hair plate organ is sensitive both to mechanical and to chemical stimuli.

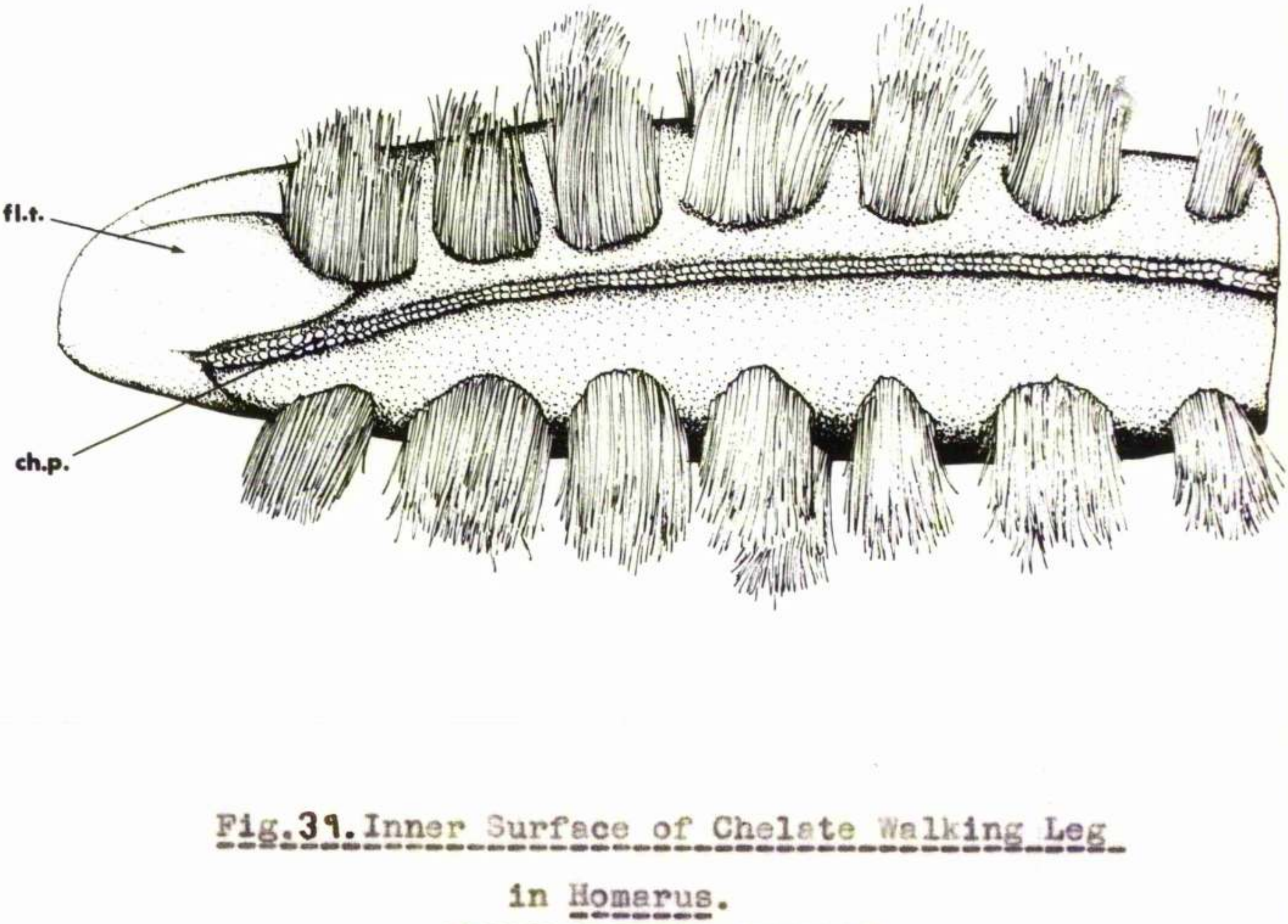


Fig.39. Inner Surface of Chelate Walking Leg in Homarus.

b = Bunch of Mechano-receptor hairs.

ch.p. = Chemoreceptor hair plates.

fl.t. = Flexible pad at leg tip.

Electrophysiological evidence tends to support the latter idea since the spike heights of responses to mechanical and to chemical stimuli are the same (Fig.40). Purely mechano-sensory units normally give rise to spikes three or four times larger than those recorded from the hair plate organs. Fig.41 shows a response to chemical stimulation from three hair plate organs.

Structure of Hair Plate Organs.

Each hair plate is a short stout hair which is covered on its proximal side with two to three hundred branches. Each hair plate is innervated by a similar number of fine nerve fibres (Fig. 42&43) and in some preparations it is possible to see that a dendrite enters the base of each branch and travels up it for some distance.

There is no evidence for any innervation of the basal region as occurs in tactile hairs so that it seems clear that each hair is innervated by one class of neurones only and that the dendrites of these neurones respond both to chemical and to mechanical stimuli.

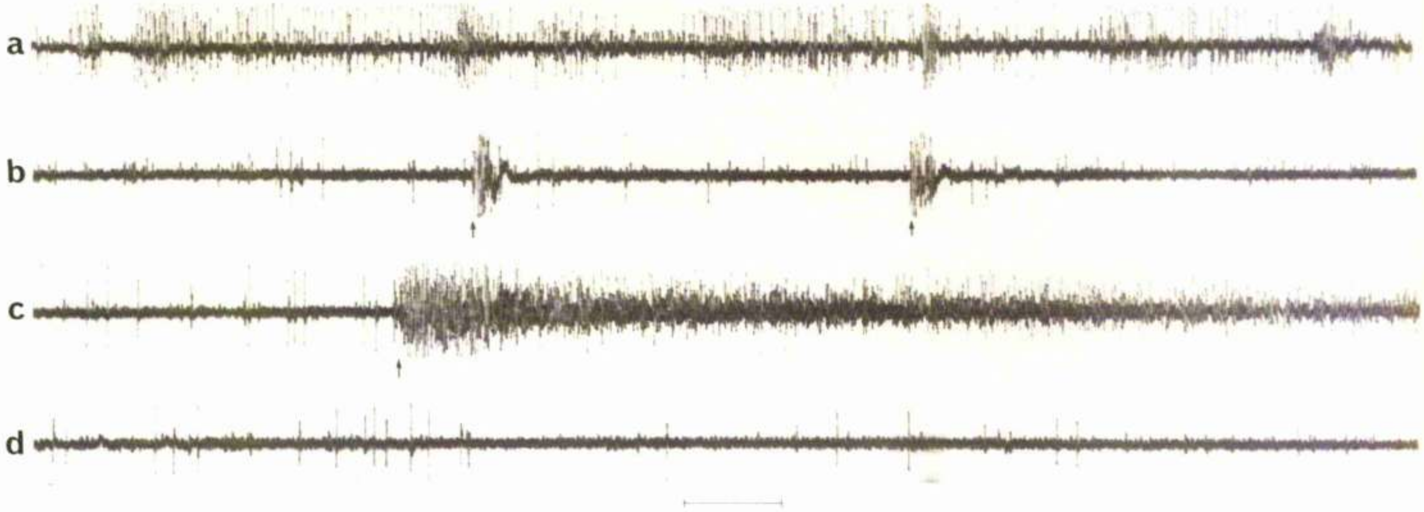


Fig. 40. Chemoreceptor Activity in the Hair Plate Row of a Homarus Chelate Walking Leg.

Despite innervation by one class of neurone only, these Hair Plates are both mechanically and chemically sensitive.

a) Response to stroking with a probe.

b) Response to 2 drops of sea water.

c) Response to I drop of filtered *Mytilus* extract.

d) Background after sea water rinsing.

TM = I sec.

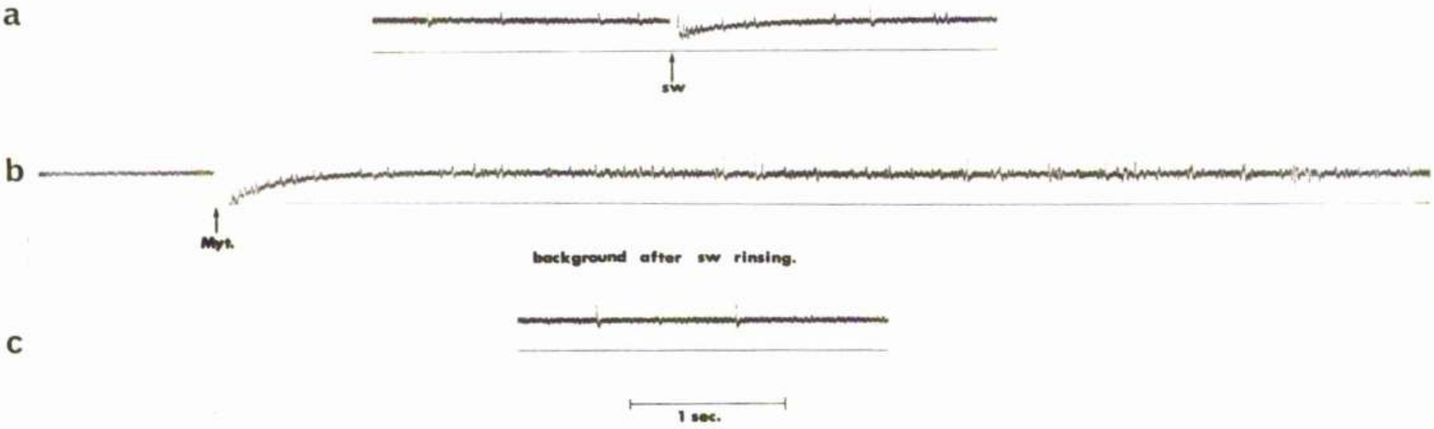


Fig.41. Responses from a Group of Three Hair Plates (*Homarus*).

a) Response to one drop of sea water.

b) Response to filtered *Mytilus* extract.

c) Background after sea water rinsing.

TM = I sec.

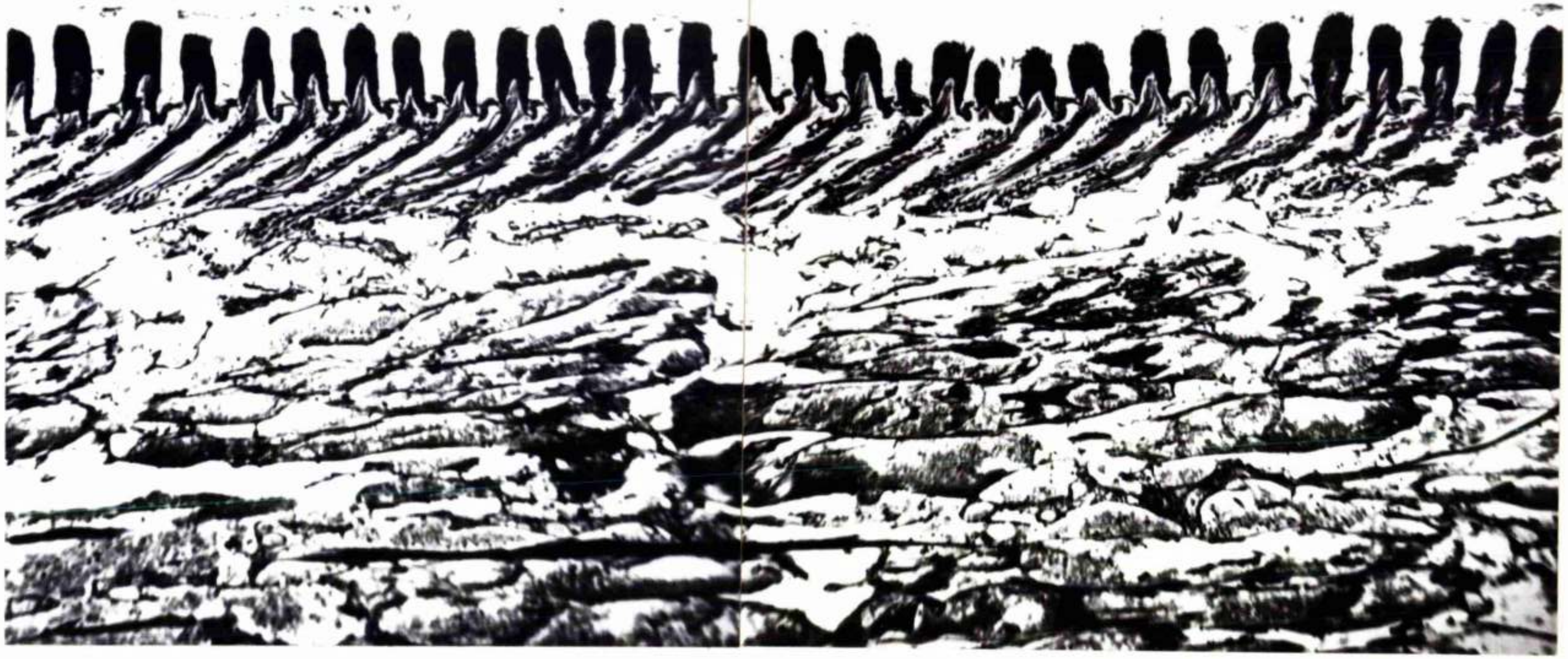


Fig.42. Row of Hair Plate Organs from *Homarus* Chelate Walking Leg.

These plates are the anatomical basis for the contact chemoreceptor sense present in these appendages.

Arrow points distally.

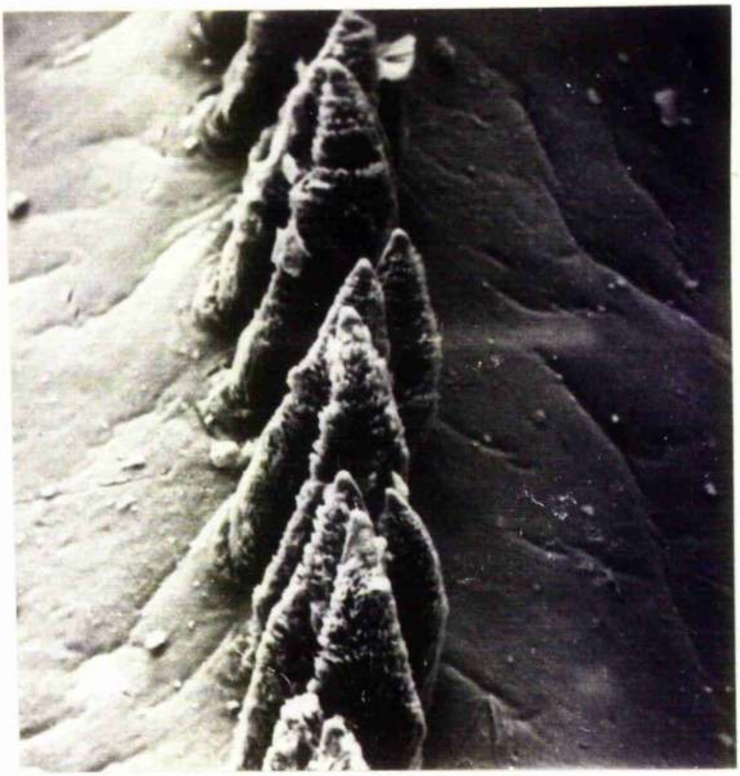
Fig. 43. A Single Hair Plate.

This illustrates the branching of the hair plates. There is a rough correspondence between the number of fine dendrites entering the base of each hair and the number of branches.

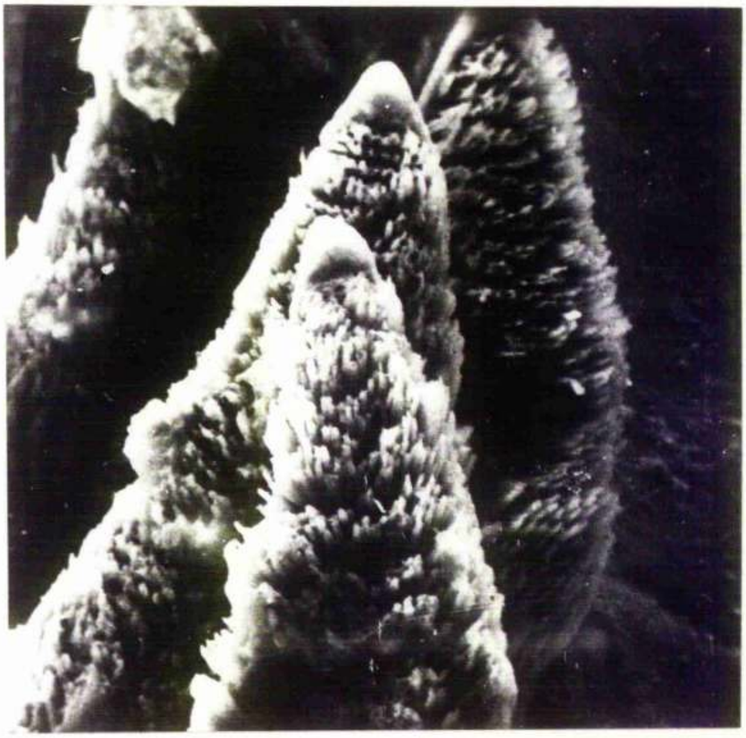
The detailed surface structure of these hair plates is the subject of Fig.44 & 45. The fine branches are largely restricted to one side of the hair and the apical region is completely free of branching. Because each hair plate is set at a slight angle and possesses a definite curvature bending the hair plate causes the branches to spring up like the spines of a hedgehog (Fig.46). This means that the maximum possible area of chemoreceptor branches is in contact with any external object gripped by the small chela. This is illustrated by Fig.47. It is interesting to recall Cowles' (1908) observation that the Sand Crab *Ocypoda arenaria* which tests its food by pinching it first in its chela and only then conveys it to the mouth. Almost certainly hair plate organs would have been the sense organs involved.

Chemoreceptor Hairs on the Large Chelae.

Although the inner surfaces of both chelae are sensitive to chemical stimuli it is in the cutter claw that the



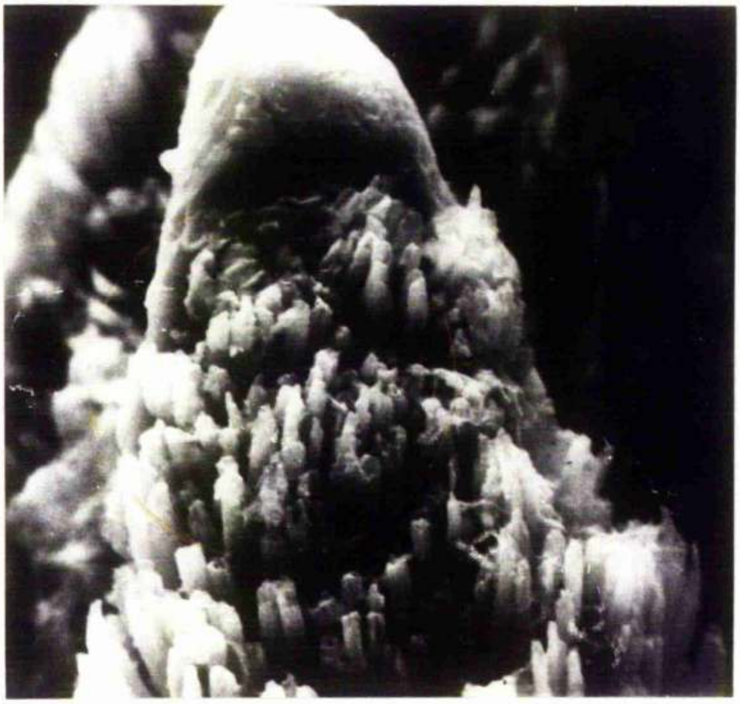
X 132



X 390

Fig.44

Surface structure of the row of hair plates which lines both inner surfaces of the chelate pereiopods of Homarus.



X 1,320

Fig.45.

Surface structure of a hair plate.

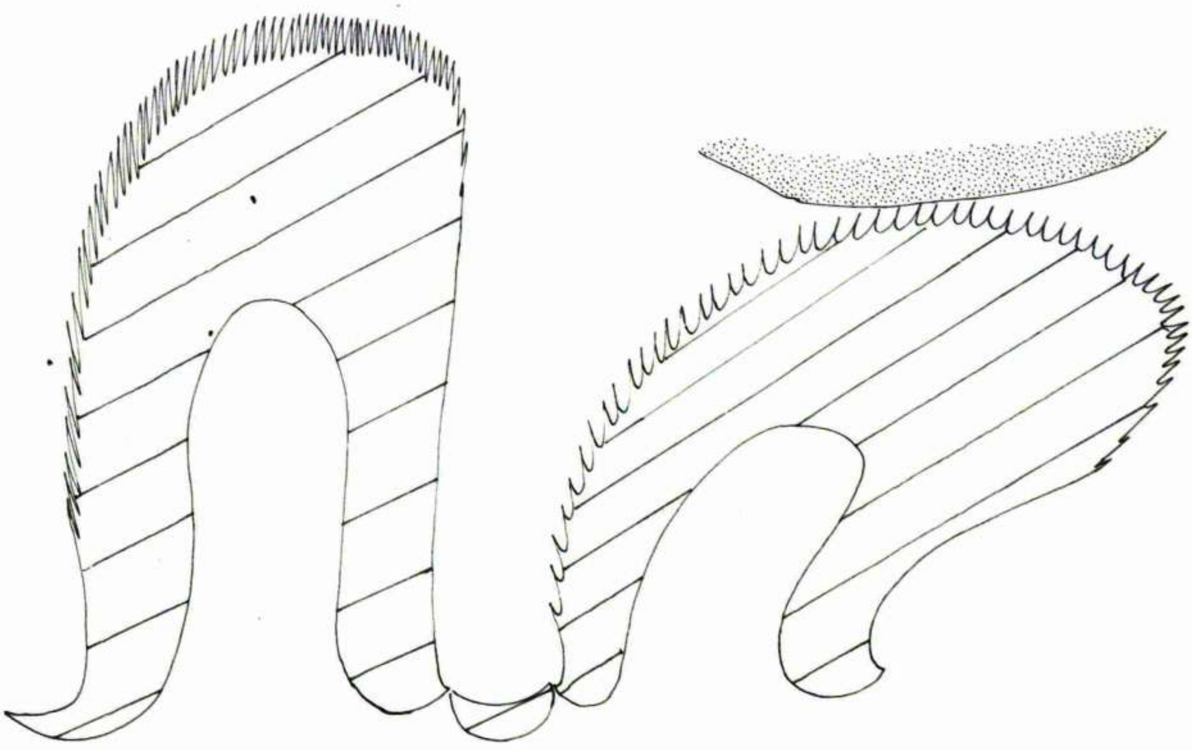


Fig.46. Presumed mode of action of Hair Plate.

Bending of the hair plate when the chela closes on an object causes the erection of the chemo-sensory branches which are thus fully exposed to any substance emanating from the object gripped.

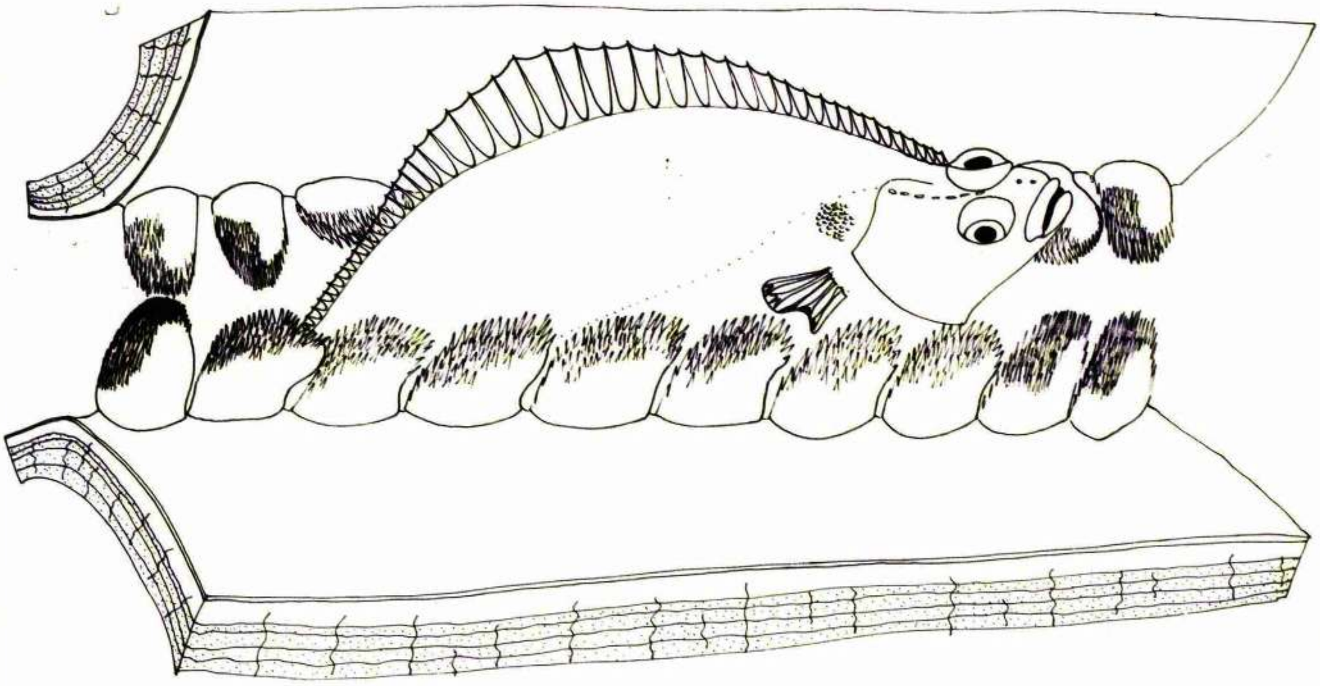


Fig.47. Action of Hair Plate Row.

This illustrates the action of the hair plate row as a whole when an object is grasped by a small chela. Erection of branches occurs whenever a hair plate is bent by the object grasped (Fig.46).

chemical sense is most developed. Small clumps of branched hairs occur on either side of the cutting edge (Fig. 48 & 49) and it is here that the chemical sense of the chelae resides. Nervous activity can only be recorded from fibre bundles innervating these hair bunches and the claw closing reflex to chemical stimuli only occurs in response to the stimulation of the chemo-sensory hairs. There is no evidence that the cutting edge itself is sensitive to chemical stimulation. Nor is there any evidence for fine dendrites with undifferentiated endings in the cuticle of the cutting edge. Campaniform organs are the only innervated structures present here. The presence of this central cutting edge distinguishes the large chelae from the smaller claws on the first two pairs of pereiopods so that objects picked up by the large claw are not directly gripped by chemosensory hair plates. The hair bunches are subjected to chemical stimuli as the cutting edge of the large claw grasps and shears through the object

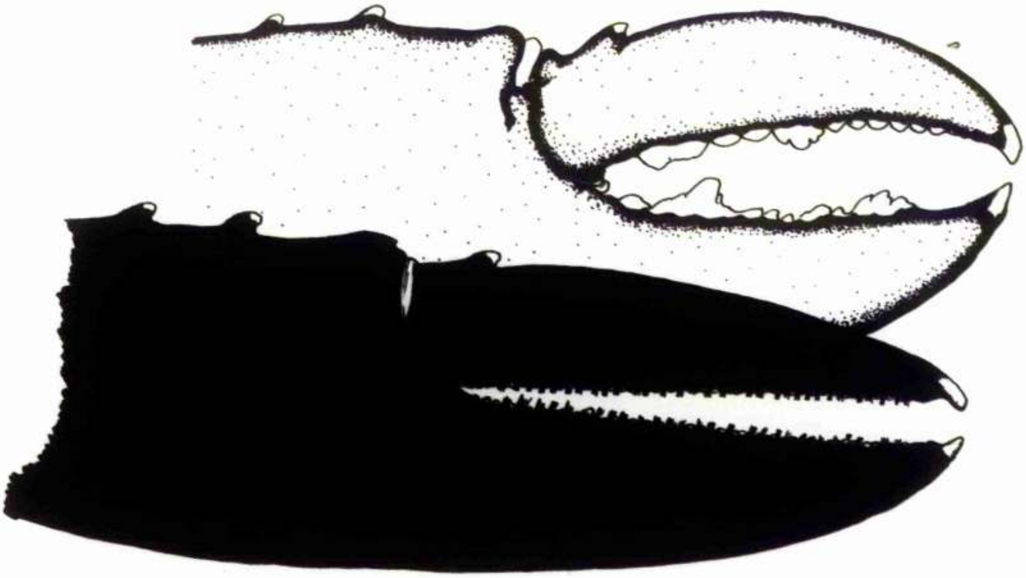


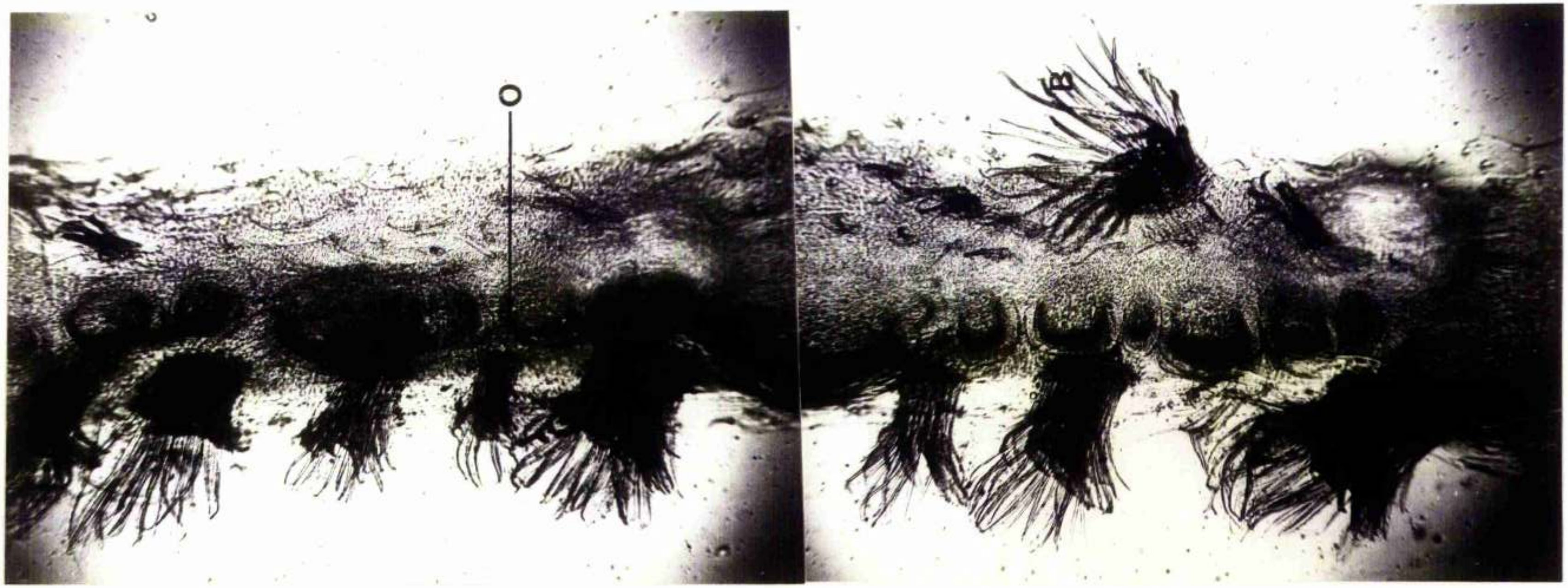
Fig.48. Chelae of Homarus vulgaris.

The chelae of this species are not identical but are differentiated into "cutter" and "crusher" claws. However, the "cutter" claw (nearer) is liberally supplied with chemoreceptor hairs along either side of the cutting edge and could equally well be called the "taster" claw.

A small number of similar hairs are also present on the crusher claw.

Fig.49. Cutting Edge of Cutter Claw.

The edge itself is formed of a row of epicuticular ossicles (O). On either side of the edge are aggregations of chemosensory hairs.



in its grip. The elaborate structure of the hair plate organs of the small chelae is lacking in the hair bunches of the cutter and crusher claws. The structure of these hairs resembles the longer, branched, chemosensory hairs of the non-chelate walking legs.

Chemoreceptor Organs of the Mouthparts.

The mouth region of Decapod Crustacea is surrounded by six pairs of highly differentiated appendages which together form the mouthparts. The first three pairs of these appendages, namely the mandibles, maxillules and maxillae are considered to be part of the head. The other pairs of appendages associated with the mouth, the three pairs of maxillipeds, are thoracic in origin and are intermediate in structure and function between the mouthparts proper and the walking legs and chelae.

All six pairs of mouthparts are involved in some way with handling external objects which are later to be introduced into the mouth. However, the mouthparts are also concerned with various other functions such as maintaining and directing the respiratory current.

We are concerned here only with the food handling part of their function and the sense organs involved in it.

The Maxillipeds.

The third maxillipeds are the longest pair of mouthparts (Fig.50) and have two main functions.

(I) Cleaning the antennules after chemical contamination (Fig.51).

(2) Picking objects off the sea floor and passing them to the second maxillipeds.

The second and first maxillipeds are concerned in handling the food and guiding it to the three pairs of short, head appendages which immediately surround the mouth.

Three main sensory structures are present on the maxillipeds, campaniform sensillae, large mechanoreceptor hairs and fine comb hairs (Fig.52 & 53) which respond strongly to chemical stimuli (Fig.54). These appear to be the only chemosensory structure represented on the maxillipeds. Each hair has a tanned basal shaft (t in Fig. 53) and an untanned distal region with a variable number of opposite, comb-like branches. These hairs are used to comb the aesthetasc and guard hairs of the antennules after contamination with small food particles. The third maxillipeds can thus taste whether the antennule chemoreceptors are clean or not. Since adaptation to chemical stimuli occurs comparatively quickly the antennules presumably require the double check of the maxilliped comb hairs in order to ensure their cleanliness.

The third and second maxillipeds have a serrated inner edge much like the cutting edge of the cutter claw. The first maxilliped possesses a single row of stout spines on its inner surface behind which are a sparse population of mechanoreceptor and comb hairs, (Fig.55).

The maxilla and the maxillule possess rather smaller gripping spines and a small number of comb-hairs (Fig.56 upper). The mandible is the innermost of the mouthparts and largely consists of a massive basal sclerite with a toothed biting edge containing campaniform organs. Chemosensory structures are not represented on the basal body of the mandible but

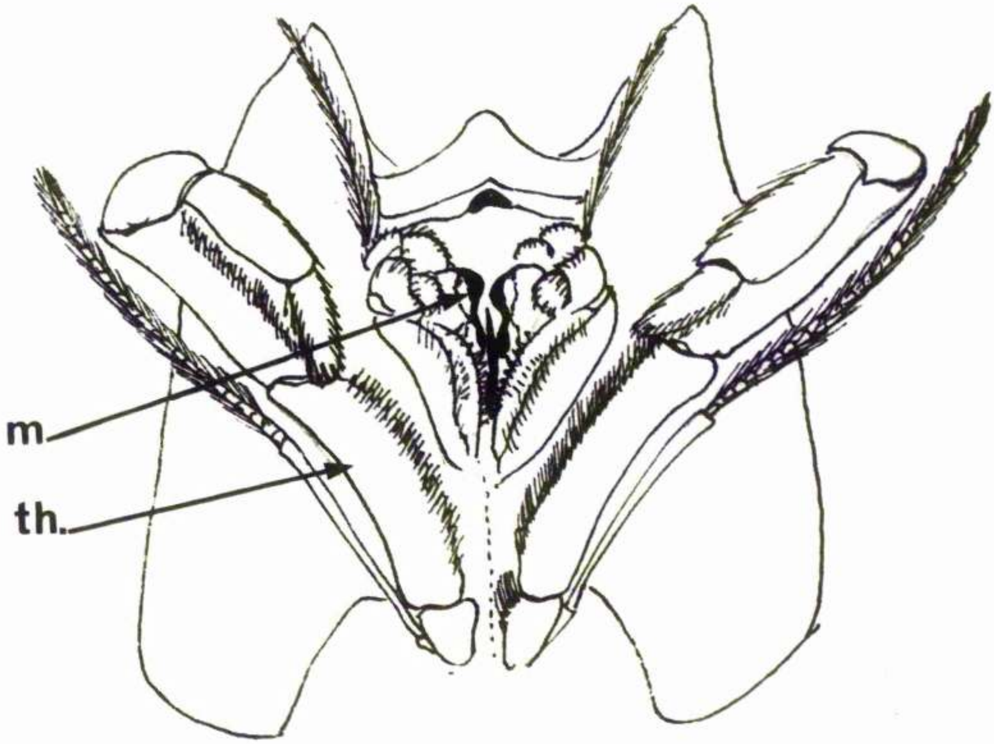


Fig.50. Mouth of Homarus vulgaris.

Between the third maxillipeds (th) and the mandibles (m) are four other pairs of appendages which sort and guide food to the mouth aperture itself.

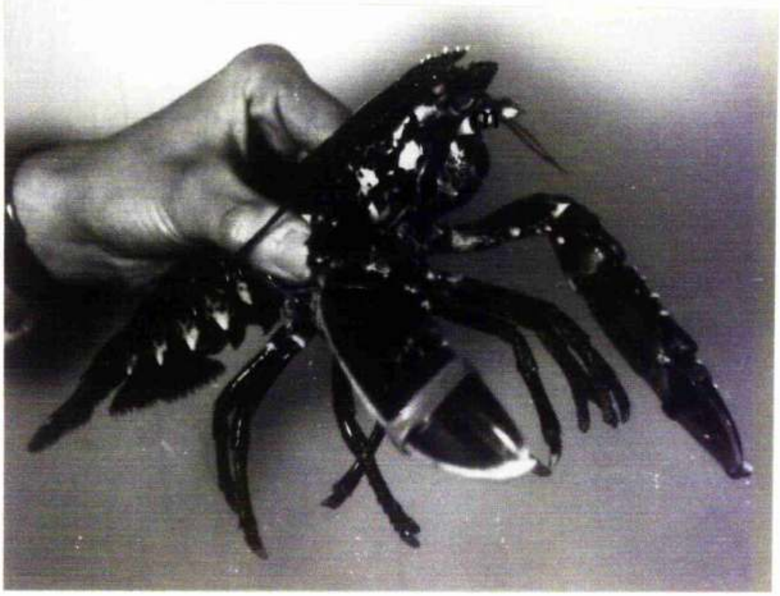
Fig. 51. Antennule Cleaning Reflex.

A) Antennules free.

B) After chemical stimulation the antennules (a) are then cleaned by the branched chemo-receptor hairs on the 3rd. maxillipeds (m).

A

B



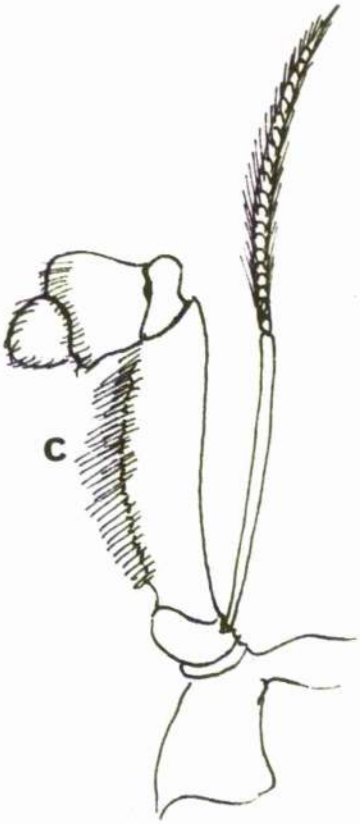


Fig.52. 2nd.Maxilliped (H.vulgaris).

The inner edge is composed of a row of epicuticular ossicles. Comb hairs (C) are present on either side of these ossicles.

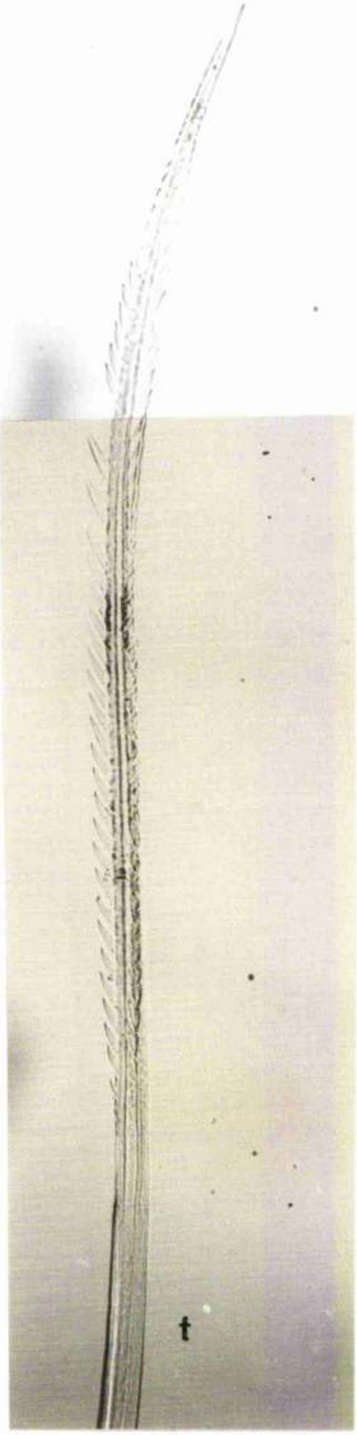


Fig.53. Comb Hair of *Homarus vulgaris*.

The distal region of these chemosensory hairs is translucent and untanned. The proximal, unbranched regio only exhibits tanning (t).

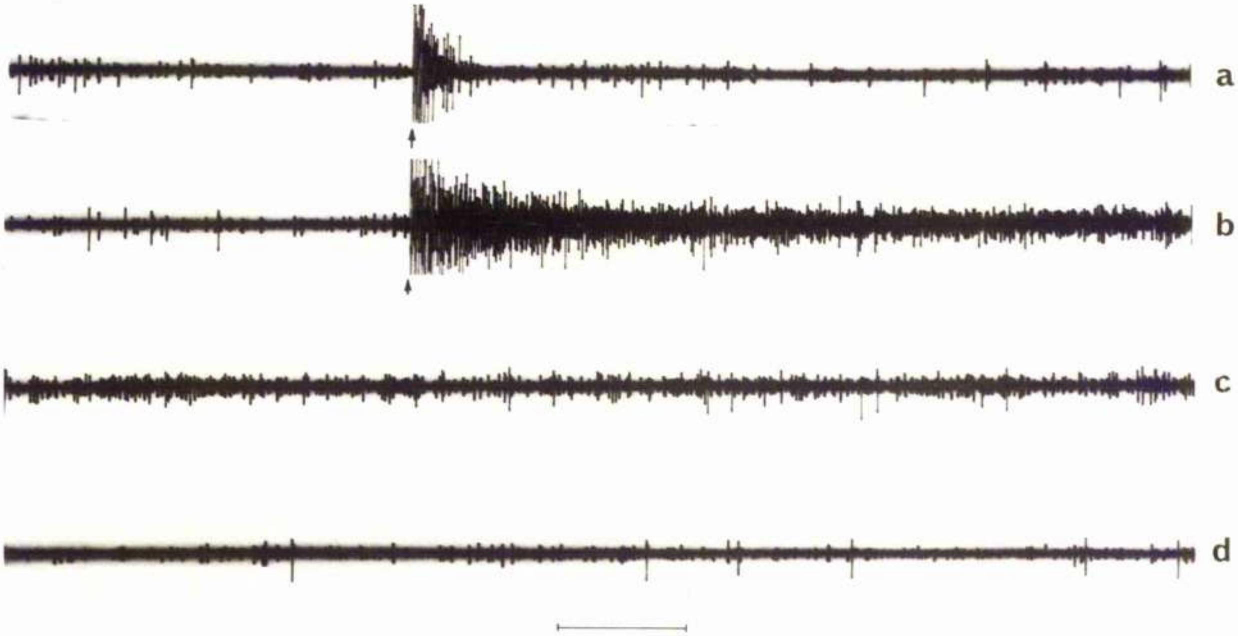


Fig.54. Chemoreceptor Activity from the Comb Hairs of the Maxillipeds (*Homarus*).

a) Response to one drop of sea water.

b) Response to one drop of filtered *Gadus* extract.

c) Response after 20 seconds.

d) Background after sea water washing.

TM = I sec.

The comb hairs are used to clean the aesthetasc hairs of the antennules after chemical contamination.

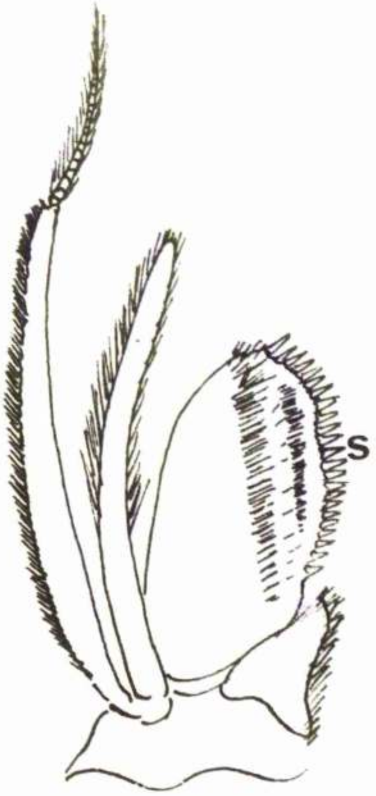
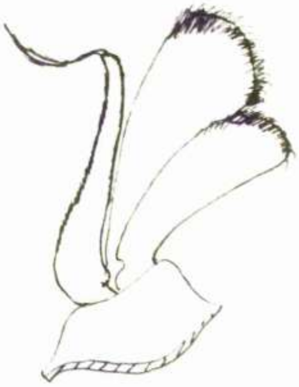


Fig. 55. Ist. Maxillired of Homarus vulgaris.

The inner edge of this appendage is armed with a row of spines (S). Comb hairs are present in two successive outer rows.



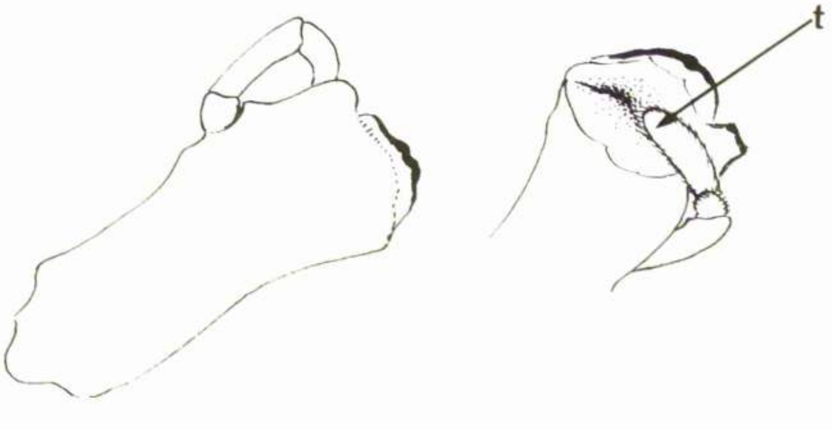


Fig.56. Maxillule and Mandible of Homarus vulgaris.

Both appendages possess comb hairs. In the mandible these are restricted to the small, tasting palp (t).

a thick fringe of comb hairs is borne on the mandible palp. This palp is normally held in the cavity proximal to the incisor process (Fig.56 lower). In the intact animal this palp is on the buccal side of the mandible and is effectively the animal's last opportunity to test its food before it is thrust into the short oesophagus and on into the gastric mill. It is possible that the palp is also used to clean out the back of the mandible after feeding.

Conclusion.

We now know that a variety of chemoreceptor organs occur in Decapoda in addition to the aesthetasc hairs of the antennules. The latter are unique in being the only unbranched chemoreceptor hair known in Crustacea. All the other chemoreceptor organs described here exhibit branching in various degrees. It is, perhaps, misleading to describe the aesthetasc hair as unbranched as it possesses a thin terminal region which could be regarded as a single branch. The aesthetasc hairs are subjected to a very considerable degree of irrigation during the flicking reflex of the antennules and the comparatively small surface area of an aesthetasc (due to the lack of branching) is presumably sufficient for adequate functioning provided a large quantity of sea water is passing each hair per unit time.

The comb hairs of the mouthparts possess a fairly large degree of branching and are subjected to a limited amount of irrigation during the waving movements of the maxillipeds that take place during the feeding behaviour.

The pereiopod chemoreceptors are very limited in the amount of sea water which they sample per second and the large degree of branching characteristic of these hairs is possibly correlated with this. It is the hair plate organs and the chemosensory hairs of the large chelae which show the greatest degree of branching. These organs are the only real contact chemoreceptors in Decapoda so far as is known at present and are subjected to virtually no irrigation.

We are led to conclude that clear cut distinctions between contact and distance chemoreceptors in Crustacea are very difficult to draw when so many intermediate types exist. Nevertheless, it does seem reasonable to assume that a negative correlation exists between the amount of irrigation to which a chemoreceptor hair is subject and the degree of branching of that hair.

THE CHEMORECEPTOR SYSTEM IN FEEDING BEHAVIOUR.

Feeding behaviour in Decapoda is a complex series of reflexes in which all the chemoreceptor types described above play a part.

Stimulatory substances are picked up first of all by the aesthetasc hairs on the antennules as they flick through the respiratory current. The three pairs of maxillipeds then execute waving movements thus irrigating their comb hairs and increasing the chances of picking up stimulatory molecules and the Lobster or Crab commences exploratory walking which usually leads the animal, often by a tortuous route, to the immediate vicinity of the source of stimulatory chemicals. In the case of Lobsters the food is usually then touched by the antennules which are afterwards combed clean by the third maxillipeds. The short antennules of crabs have a much higher flicking rate than Lobsters and are presumably self cleaning. In any case they are not combed by the third maxillipeds.

The chemoreceptors of the walking legs and chelae are then brought into play as the food is directly touched and gathered up to the mouthparts. From then on the food is guided into the oesophagus and a certain amount of preliminary cutting up takes place as the polychaete or small fish is pushed past the mandibles.

The Visual Sense in Feeding Behaviour.

Although chemical stimuli play a very important part in feeding behaviour it is perhaps little realized that visual stimuli are also important in some feeding situations. Both Lobsters and Crabs are capable of catching small fish with their chelae and at times when bait is scarce commercial fishermen successfully use broken white crockery in their Crab or Lobster pots.

DISCUSSION

Like most studies of very incompletely known animals this work has posed almost as many questions as it has answered. Taking the latter first we now know that the Crustacean chemoreceptor system is based on hair-sensillae and that the "funnel canal organ" of Luther (1930) is a type of campaniform organ. This is knowledge which has been gained by using electrophysiology as a histological tool and thus building up a picture of the functional anatomy of identified structures.

It is probable that with this work and that of Pabst and Kennedy (1967) all the major types of Decapod cuticular sense organ have now been functionally described. The early anatomical literature is adequately summarized in Bullock and Horridge (1965) and indicates an enormous variety of innervated cuticular structures. It is this author's belief that this is something of a false picture of Malacostracan cuticular sense organs. Small intra and extraspecific variations in anatomical detail tend to confuse an overall concept of a relatively small number of basic functional types which are themselves inter-related.

(I) Hairs which are not innervated.

By no means all hairs are sensory and possess an innervation. The ccampanion hairs described by Mellon (1963) from the thorax of the freshwater crayfish are typical of this class. Some of these hairs show an extreme degree of fan-like branching as in the fringe hairs of the maxilliped flagella and the border of the carapace. Others are extremely simple in structure, for example, the large numbers of unbranched, non-innervated hairs which occur inside the gastric mill. In short, there is a considerable variety of non-sensory hairs in Crustacea all of which are employed in mechanical or hydrodynamic roles.

(2) Mechanoreceptor Hairs.

All innervated hairs appear

to exhibit some sensitivity to mechanical stimulation. In this category we are excluding hairs which are primarily chemosensory in function.

Mechanosensory hairs are typically innervated by a small number of fairly large neurones, the dendrites of which are inserted either at the base or some distance up the shaft of the hair concerned. Many of these hairs have a dual innervation (Mellon I963, Laverack I964) and respond differentially to movement in different directions.

There is considerable variation in the cuticular structure of the hairs themselves. Most of the larger types show at most a sparse terminal branching but the smaller types are squat and heavily branched. To this latter category belong the Buschelorganen of Luther (I930) and the Hair-fan and Hair-peg organs of Laverack (I962 a&b).

If it is possible to speak of a basic primitive Crustacean cuticular sensillum then the mechanoreceptor hair is surely that and within this type possibly the least specialized are those hairs which possess more than two neurones, the dendrites of which pass some distance up the hair shaft.

(3) Chemosensory Hairs.

All known varieties of chemosensory hair possess a multiple (often over 200 neurones per hair) innervation of small bipolar nerve cells. Most types are branched, there being a negative correlation between the surface area (degree of branching) of the hair and the amount of irrigation to which it is subject. All available evidence indicates that all the neurones associated with these hairs belong to one class and respond both to chemical and mechanical stimuli. The branches of these hairs are typically untanned. How the chemical stimulus reaches the dendrite remains a problem and while it is conceivable that these substances can pass through the untanned cuticle Laverack and Ardill (1965) found no evidences for pores through the cuticle of the aesthetasc hairs of *Panulirus argus*. However, the dendrites are apparently exposed to the external environment via a large pore at the end of the hair and it is thus possible that chemical transduction takes place here.

(4) Campaniform Organs.

Campaniform organs are small mechanoreceptors which respond to strain applied to the cuticle in which the organ is situated. The structure of these organs is simple and constant. Two or more dendrites with a cuticular sheath are inserted into a cap-like disc of flexible cuticle which is distorted when the surrounding cuticle is strained. It is possible to interpret campaniform organs as highly modified hair sensillae from which the shaft of the hair has been lost. The cap resembles the basal region of mechanosensory hairs in consisting of a flexible, resilin-like cuticle.

Campaniform organs exist in two main situations:
(I) As universally distributed receptors for externally applied cuticular strain. These are normally innervated

by two or more small bipolar neurones.

(2) As distinct aggregations of dually innervated large organs which are either part of proprioceptor organs ("slit sensillae" of *Homarus*, Wiersma 1959) or as a compound tactile/proprioceptor organ at the tips of the pereiopods.

There was no evidence in this work for the existence of free nerve endings in hard cuticle as described by Wetzel (1935) for the mandibles of *Caprella*. In all cases where this apparently occurred closer examination always revealed the presence of the typical cap and peg components of campaniform organs at the dendrite tips. Wetzel (1935) also described branching in these free cuticular endings as did Pringle (1938 I) for the campaniform organs of *Periplaneta*. Absolutely no evidence for this was observed in this study.

Pabst and Kennedy (1967) have recently described mechanically sensitive nerve endings from the arthrodial membranes of Decapoda but give no details of the structure of the nerve endings. It will be very interesting to see whether these prove on closer histological examination to be another category of campaniform organ.

Eyes.

It is possible that in the future information will be amassed which will enable simple and compound eyes to be included in a general classification of inter-related Crustacean sense organs. However, this is not yet possible and eyes are thus excluded from this simple scheme.

Inter-relationships between Cuticular Sensillae.

It is at this point that we must inevitably pass from the realm of questions answered to the realm of questions asked. If the mechano-receptor hair with dendrites passing up the inside of the shaft be taken as a basic starting point chemoreceptor hairs can be derived by a process of elaboration, in which the dendrites are multiplied and extended to innervate branch or terminal regions at which the dendrites are exposed to the external environment. The mechanical sensitivity of chemoreceptor hairs is presumably due to the stretching or compression of nerve endings when these hairs or their branches are deflected. It seems likely that the dividing line between chemosensory hairs and the less specialized mechanically sensitive types is by no means clear cut and it is possible that this latter type is sensitive to moderately extreme chemical stimuli. The question remains open.

The possibility that campaniform organs are derived from highly reduced mechanosensory hairs has been discussed above. The view has also been expressed in this thesis that campaniform organs may be the cuticular sense organ from which sub-cuticular chordotonal organs have been derived. The apparent retention of the cap and peg structure in the chordotonal organs described by Taylor (1967) highlights the possibility that not all biological structures are necessarily functional but may merely be an expression of the evolutionary origin of the structure involved. This concept of vestigial structures is well-known in the tradition of vertebrate zoology. The increasing post war participation of physical scientists in biological research has led to great advances in the understanding of biological mechanisms in terms of mathematical and chemical models. Nevertheless, there is the ever present danger that ignorance of the value of the comparative approach to the physiology of invertebrates can lead to the production of elaborate theories "explaining" the mechanism of structures which are actually non-functional evolutionary leftovers.

Problems still unsolved.

While the scanning electron microscope provides a fairly satisfactory means of examining the superficial structure of the cap region of campaniform organs any theory of their transduction mechanism must remain preliminary until sections of the dendrites and their endings in the peg region at its insertion in the cap have been examined in the electron microscope. This has so far proved impossible for the large Decapoda used in this study. However, now that it is possible to recognise Crustacean campaniform organs anatomically it is to be hoped that the electron microscopy of campaniform organs may be feasible for Zoaea larvae.

Transduction mechanisms in Crustacean chemoreceptors are also largely a mystery and there is certainly a need for more work on their fine structure. The difficulty of fixing soft nervous tissue inside a hard hair shaft is a real difficulty here and this can lead to the contraction of the soft inner tissue down the outer shaft. On top of this is the ever-present problem of cutting thin sections of hard tissues. Once again work on small larvae may be the answer here.

The Problem of Pressure Reception.

Most planktonic organisms respond to pressure changes and Decapod larvae are no exception (Hardy & Bainbridge I95I).

There are a number of theories seeking to explain the sensory basis for the pressure response and it is interesting to consider whether any of the sense organs discussed in this work could act as pressure change detectors.

Hardy and Bainbridge (1951), bearing in mind the low compressibility of sea water, suggested that small gas vesicles might be the basis for pressure sensitivity in Decapoda. However, subsequent work (reviewed by Knight-Jones & Morgan 1966) is against this idea. No gas vesicles have ever been seen in marine Crustacean larvae and the characteristics of the pressure response which is just as sensitive at very high as at very low pressures is strong evidence against the gas vesicle idea.

A characteristic of the response to pressure is the apparent adaptation which takes place (Rice 1968, personal communication). A large increase in pressure will cause the larva to swim upwards. However, swimming stops long before the depth zone corresponding to the original pressure is attained. If at this point a small extra pressure increase be applied an upward migration takes place almost equal to the response to the large pressure change originally applied. Adaptation of this kind is typical of most sense organs both chemical and mechanical and it is probably due to a failure to maintain the generator potential.

Although the soft parts of Crustacea are relatively incompressible the hard parts are a lot more so. It is thus to be expected that amplified, though still minute, movements would occur at arthrodial membranes. Possibly the endings described by Pabst and Kennedy (I968) would be sensitive to these small movements. Similarly, a compressible hypodermal cell beneath the cap of a campaniform organ could enable such an organ to act as a pressure receptor. Digby (I96I) found that the potential difference across the surface of *Praunus* varied with changes of pressure and suggested that this could be the basis of the pressure sense. The potential change involved is quite large (IOmV for a three atmosphere change in pressure). It remains to be shown whether Crustacean sense organs exist which would respond to this. It is quite conceivable that chemoreceptor hairs would respond under these conditions. In any event all existing evidence suggests that it is not an undescribed cuticular sense organ which mediates the pressure response but an already well known sensory structure which can also act in a pressure sensitive role. While we may well, by now, have described all the major categories of Decapod sense organs it will surely be a long time before all the physiological potentialities of the various sensilla have been explored and explained in terms of the fine structure of the organs involved.

SUMMARY

(I) Evidence is presented that the "Funnel Canal Organs" described by Luther (I930) are not contact chemoreceptors.

(2) Experiments are described which indicate that the Funnel Canal Organ is a mechanoreceptor which responds to strain applied to the cuticle.

(3) Histological observations suggest that these sense organs are actually campaniform sensilla which occur both singly and in groups, to form compound sense organs.

(4) The relationship between campaniform and chordotonal organs is discussed.

(5) Further experiments show that a large variety of chemoreceptor hairs exists. Most are branched and there is a negative correlation between the

surface area of the hair (degree of branching) and the amount of irrigation to which the hair is normally subject.

(6) The view is expressed that the concept of contact chemoreception is of limited use when considering aquatic Crustacea. The Hair Plate Organs of the chelate pereiopods are suggested as the only chemoreceptor organs in aquatic Decapoda which fulfill a true contact role.

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