ANATOMY AND PHYSIOLOGY OF ORGANS INVOLVED IN FOOD INGESTION IN THE LOBSTER, HOMARUS GAMMARUS L.

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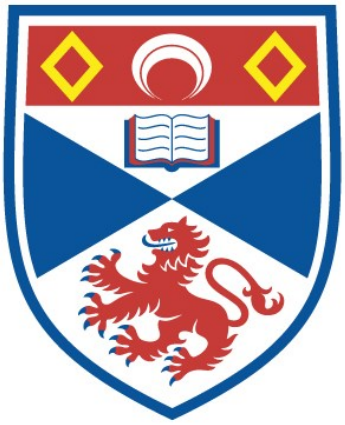

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THE ANATOMY AND PHYSIOLOGY OF ORGANS INVOLVED IN FOOD INGESTION IN THE LOBSTER, *Homarus gammarus* (L.)

by

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ABSTRACT

The dissertation describes the gross neuromuscular anatomy of the labrum (upper lip) and oesophagus of the lobster *Homarus gammarus* as a pre-requisite for studies on the mechanisms and control of food ingestion. Sense organs of the area are also described. Of particular interest are two paired sensors (the anterior and posterior oesophageal sensors) which are bilaterally situated at the oesophageal/cardiac sac valve. These are similar to contact chemoreceptors previously described in insects, and are classified as such on morphological grounds and with indirect electrophysiological evidence.

The labrum undergoes rhythmical retraction/protraction movements during feeding and can be shown to participate in both the mandibular rhythm and oesophageal peristalsis. Its role in feeding is discussed. Subsequently, small labral protractions are used as an indication of the duration and frequency of oesophageal peristalsis.

Oesophageal peristalsis is effected by the co-ordinated contraction of the oesophageal musculature. This is controlled by rhythmical bursting neuronal activity which can be recorded from the nerve trunks in the area. A characteristic burst recorded from the superior oesophageal nerve is used as an indication of oesophageal dilatation during peristalsis for studies on the feedback effects of the oesophageal sensors.

Electrical and chemical stimulation of the posterior oesophageal sensors can initiate and increase the frequency of oesophageal peristalsis, while stimulation of the anterior oesophageal sensors can slow and terminate oesophageal peristalsis.

The results are discussed and, in conclusion, a model of the role of the oesophageal sensors in feeding is presented.

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A thesis presented for the degree of Doctor of Philosophy at the University of St. Andrews

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March 1978

SUPERVISOR'S CERTIFICATE

I certify that R.M. Robertson has fulfilled the conditions laid down under Ordinance General No. 12 of the University of St. Andrews and is accordingly qualified to submit this thesis for the degree of Doctor of Philosophy.

DECLARATION

I declare that the work reported in this thesis is my own and has not been submitted for any other degree.

VITAE

I was educated at Trinity College, Glenalmond and attended University at St. Andrews where I graduated in Zoology in 1973. The work described in this thesis was carried out between October, 1973 and December, 1976.

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CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Rhythmical motor activity plays an important part in the lives of most animals. It controls such diverse processes as respiration, feeding, circulation and locomotion. In recent years, since the advent of sophisticated intracellular recording techniques, the study of the neuronal mechanisms underlying such activity has increased. This is partly because such systems control these vital processes and partly for the pragmatic reason that the rhythmicity and repetition provide large samples of data for analysis (Macmillan, 1977). Thus an insight can be gained into the control of some behavioural events. The studies to date have tended to centre on three main questions. Firstly, what is the nature of the central oscillator? Secondly, what are the relative roles of central programming and peripheral feedback in determining an action pattern? Finally, how are such behaviours turned on, turned off and modified from higher nervous centres. The purpose of this dissertation is to describe the rhythmical movements of the mouth, the labrum (upper lip) and the oesophagus of Homarus gammarus during feeding and to make an exploratory study of their control. As an introduction to provide the background for the work reported here, brief reviews on the above three topics will be presented here. They are restricted to studies on invertebrates, and no attempt has been made to make them exhaustive. Those articles which are quoted simply serve to illustrate some established principles.

The Nature of Central Oscillators

The present convention is to divide oscillators into two main types (Moffett, 1977); endogenous oscillators in which the intrinsic properties of a single neuron act to set up and maintain an oscillating membrane potential; and connectivity oscillators in which the electrical and chemical synapses within a pool of two or more neurons can produce rhythmicity without the provision of any external timing cue. The latter group is further divided into network connectivity oscillators (a specific

synaptic network forming a circular pathway), and electrotonically coupled oscillators (a system of electrotonically coupled neurons whose membrane properties interact to form a rhythmically active unit).

(a) Endogenous Oscillators

These are individual neurons whose membrane potentials continually oscillate within defined limits. This can be experimentally induced in silent neurons, the better to study the ionic mechanisms underlying such activity; for example by maintaining the neurons in a medium in which the calcium is replaced by barium (Gola, Ducreux and Chagneux, 1977). There are currently two theories to account for endogenous pacemaker activity. The first proposes that the hyperpolarisation phase during bursting activity is due to the activation of a chloride-coupled sodium pump brought about by an intracellular accumulation of sodium ions. This arises as a result of a high resting sodium conductance which causes the depolarisation phase (Strumwasser, 1967). The other hypothesis, which is similar to that proposed for the myocardial pacemaker potential, suggests that the hyperpolarising phase is a result of the activation of a slowly inactivating potassium conductance. The slow inactivation of this current results in a depolarisation which triggers a voltage dependent inward current (perhaps mediated by sodium) to cause rapid depolarisation and bursting (Barker and Gainer, 1975a). Apart from these differences it is generally accepted that an inherent instability of the membrane potential is best evidenced by a negative slope region in the steady-state current/voltage relationship (Smith, Barker and Gainer, 1975; Gola, Ducreux and Chagneux, 1977). The activity of bursting pacemakers can be significantly modified by a number of environmental and chemical factors (Ifshin, Gainer and Barker, 1975; Barker and Gainer, 1975b; Barker, Ifshin and Gainer, 1975; Cook and Hartline, 1975; Chalazonitis, 1977). Furthermore, synaptic activation at

different times during the cyclic changes of the membrane potential could alter the output of the oscillator to different extents (Chalazonitis, 1977; Ayers and Selverston, 1977; Prior and Selperin, 1977). Examples of endogenous oscillators are drivers of the pyloric rhythm of the lobster stomatogastric ganglion (Selverston, 1977); the cardiac ganglion of the spiny lobster (Friesen, 1975a,b); the salivary duct of the slug (Prior and Selperin, 1977); and a non-spiking endogenous oscillator underlies the rhythmicity of scaphognathite beating in lobsters and hermit crabs (Mendelson, 1971).

(b) Connectivity Oscillators

Electrotonically coupled oscillators can be dismissed fairly rapidly due to the lack of data concerned with them. In only one instance is an electrotonically coupled network of neurons implicated as the oscillator controlling a specific behavioural act. This is the "cyberchron" network controlling the feeding of Helisoma trivolvis, a pulmonate mollusc, (Kater, 1974), and it is thought to be identical to a similar network described in Planorbis corneus, which is closely related to H. trivolvis (Berry, 1972). That an electrotonically coupled network is capable of rhythmical bursting output as a result of a constant excitatory input has been shown by Getting and Willows (1974). However, the most common role for electrotonic synapses in oscillator systems is to ensure rapid transmission of excitation or inhibition between the individuals of a neuron pool and to promote a synchronous output (see e.g. the lobster stomatogastric system, Selverston, Russell, Miller and King, 1976).

The simplest network connectivity oscillator which one could imagine might consist of two neurons (or electrotonically coupled pools of neurons) which are coupled by reciprocal inhibitory connections and which exhibit the property of postinhibitory rebound. In principle these properties alone could account for a rhythmical alternating output in two neurons

(Perkel and Mulloney, 1974). This system is approximated for the network controlling the swimming behaviour of Tritonia diomedia (Dorsett, Willows and Hoyle, 1969 & 1973, Willows, Dorsett and Hoyle, 1973). In this case a sensory input initiates the sequence to produce alternating activity in reciprocally inhibited motoneurons which are maintained in an excited state by a regenerative feedback system. Termination of the sequence appears to be an active process. Reciprocal inhibitory connections between two groups of interneurons may comprise the oscillating element in the walking system of the cockroach (Pearson and Fourtner, 1975), and the flight system of the locust (Moffett, 1977). Postinhibitory rebound has also been implicated in the generation of the gastric rhythm of lobsters, and the feeding rhythm of Pleurobranchaea californica (Mulloney and Selverston, 1974b; Siegler, Mpitsos and Davis, 1974). The latter authors postulated that it might arise as a result of a decrease in potassium conductance and a decline in sodium inactivation as membrane polarization increases. A further property exhibited by the motoneurons controlling the feeding rhythm of Pleurobranchaea is an endogenous buretiness, perhaps resulting from an accumulative increase in potassium conductance. Although this is not required to sustain the normal rhythm, it is supposed that it may help to terminate normal bursts (Siegler, Mpitsos and Davis, 1974). Also, the metacerebral giant serotonin cells which are thought to be command elements in the feeding rhythm of Pleurobranchaea possess two membrane properties to enhance the effect of a rhythmical input. These are an accumulative post-spike conductance increase similar to that demonstrated as contributing to the buretiness of the feeding motoneurons, and anomalous rectification which acts to amplify the effect of an excitatory input during depolarisation and suppress it during hyperpolarisation (Gillette and Davis, 1977). Finally, the occurrence of non-spiking neurons with oscillatory membrane potentials

(whether intrinsically or extrinsically produced) is being found more common in the networks controlling rhythmical acts (Mendelson, 1971; Pearson and Fourtner, 1975; Simmons, 1977).

In summary: As well as the common interneuronal interactions of excitation and inhibition, either chemically or electrically mediated, the following properties are commonly found in oscillators: 1) a tendency for the membrane potential of individual neurons to oscillate, either under normal conditions or due to a steady depolarising input; 2) post-spike conductance increase and anomalous rectification to enhance the effect of a rhythmical excitatory input; 3) reciprocally inhibitory connections; 4) postinhibitory rebound; 5) electrically inexcitable membranes such that action potentials are not produced.

Central Programming and Peripheral Feedback

The relative importance of central programming and peripheral feedback in the generation of a co-ordinated rhythmical output has been a question arousing some debate in the past. Hitherto a difference of opinion existed whereby the importance of one process was exaggerated at the expense of the other. It is now generally accepted that both processes have a part to play, albeit to different extents in different circumstances (Bullock, 1961), and the main area of investigation is now in determining the precise role of the periphery in co-ordinating behavioural acts and the mechanisms which integrate sensory information into the central programme. It is often proposed that those activities which deal with a variable substrate or which operate in a heterogeneous environment are more likely to need constant sensory modulation to maintain an effective rhythm than are those systems operating under relatively fixed physical conditions (see e.g. Macmillan, 1977). An example of such a variable activity is locomotion.

Arthropod locomotory systems such as walking (Bowerman, 1977) and insect flight (Wilson, 1967), have provided invaluable preparation for these investigations. Their advantages are that the relevant sense organs are identified easily and their output can be characterised readily. There is already a wealth of data on arthropod proprioceptive mechanisms (e.g. Cohen, 1963; Finlayson, 1968; Howse, 1968; for more extensive bibliographies see Mill, 1976; Bowerman, 1977). Furthermore the fact that the action pattern is performed by a number of discrete appendages enables the contribution of their sense organs to be analysed by a variety of experimental modifications of each appendage to nullify, reduce or increase their sensory feedback (for example by amputation, use of prosthetics, ablation of specific organs, fixing of joints, loading of particular parts, and introducing spurious movements into the normal rhythm). (see e.g. Bowerman, 1975b, Macmillan, 1975). Total deafferentation without total isolation of the central nervous system is difficult, and in the latter case, rhythmicity is usually lost. It is thought that this is due to a lack of ability to activate the system (Macmillan, 1977). The development of the techniques of electromyography and high speed cinematography has enabled various parameters of a rhythmical activity (such as gait, protraction period, retraction period, step period, lag and phase, for walking; similar parameters can be analysed in other rhythms) to be analysed in both, intact and functionally modified animals. A description of the normal rhythm is necessary prior to examining the effect of experimental manipulations, and such descriptions can provide information about the underlying co-ordinating mechanisms (e.g. Bowerman, 1975, for scorpion walking; Barnes, Spirito and Evoy, 1972, for lateral walking in crabs; Macmillan, 1975, for walking in lobsters; Burrows and Willows, 1969, for rhythmic maxilliped beating in anomuran and brachyuran crustaceans; Neil, Macmillan, Laverack and Robertson, 1976, and Laverack, Neil and Robertson, 1977, for rhythmic exopodite

beating in the larval lobster and the mysid shrimp).

One of the most common effects of the stimulation of appendage chordotonal and myochordotonal organs is the production of resistance reflexes. These are reflexes which activate a muscle to oppose an imposed movement away from the initial position (Bush, 1963; Bush, 1965; Muramoto and Murayama, 1965; Bush and Roberts, 1968; Evoy and Cohen, 1969). A series of four papers on the nervous control of walking in the crab Cardisoma guanhumi has i) characterised the resistance reflexes produced by passive movement of the propo-dactylopodite and carpo-propodite joints (Spirito, Evoy and Barnes, 1972); ii) investigated the role of these reflexes in walking (Barnes, Spirito and Evoy, 1972); iii) determined the proprioceptive influences on intra and intersegmental co-ordination (Evoy and Fourtner, 1973); and iv) examined the effects of ablation of the myochordotonal organs present at the mero-carpopodite joint (Fourtner and Evoy, 1973). From this work the authors conclude that resistance reflexes do not play an important role in the co-ordination of antagonistic muscles during walking and that their primary function is to compensate for changes in the applied load as would occur during walking over irregular surfaces. In addition, input from one leg can influence the walking output of the other walking legs as well as of that leg, and the myochordotonal organs act not in response to increased load, but to determine the end point of the flexion stroke. In cockroaches flexion is terminated by excitation of trochanteral hair plates (Wong and Pearson, 1976). The role of resistance reflexes to compensate for unintended joint movement and increased load has been confirmed for Astacus walking (Barnes, 1977). It is believed that proprioceptive input from a limb is inhibited during the normal rhythm and thus resistance reflexes are not generated (Barnes, Spirito and Evoy, 1972; Field, 1974; Barnes, 1977).

Such inhibition could be mediated by a corollary discharge of the motor programme (Delcomyn, 1977). The discovery of tendon organs which respond to increases in tension provides an alternative system to mediate the reflexes produced by increased loading of a limb (Dando and Macmillan, 1973), as well as damping resistance reflexes to prevent oscillatory interactions between flexor and extensor reflexes (Macmillan, 1976). Tension afference is of paramount importance in the manipulation of different substrates by the mandibles of the lobster. Variable substrates will load the system to different degrees and a positive feedback mechanism to intensify the output and promote effective biting is necessary (Wales, Macmillan and Laverack, 1976a,b; Macmillan, Wales and Laverack, 1976).

A similar system to the resistance reflexes described above is the sensory mechanism which ensures a forceful and effective retraction of the buccal mass in the feeding rhythm of the pulmonate snail, Helisoma trivolvis (Kater and Rowell, 1973). Mechanoreceptors sensitive to buccal mass retraction have a positive feedback loop with retractor motoneurons to intensify their output. They also inhibit the protractors. The system differs from resistance reflexes in that its action is to augment and not negate the movement stimulating the sensors, apart from the fact that resistance reflexes are not generated during the normal rhythm. However, considering the role of resistance reflexes in compensating for unintended joint movements and increased load, Kater and Rowell (1973) propose that a more useful approach is to think of them as acting to intensify power strokes. Thus the reflexes can be thought of as similar in function if not in their underlying mechanisms. Clarac and Ayers (1977) describe two feedback mechanisms which co-ordinate walking in the spiny lobster, Palinurus vulgaris. Positive feedback acts to increase the discharge of the active motoneuron or inhibit the antagonist, while negative feedback is only

active at extreme joint positions and stimulates the antagonist muscle. The latter process facilitates the transfer from flexion to extension and vice versa.

It is known that rhythmical output underlying a variety of behavioural acts can be produced in totally or partially deafferented preparations (locust flight - Wilson, 1964; cockroach walking - Pearson, 1972; respiration in Limulus - Wyse, 1973; leech swimming - Kristan, Stent and Ort, 1974; leech heartbeat - Thompson and Stent, 1976a; pyloric rhythm of lobsters - Selverston, 1977). However, it is also known that this basic, centrally-generated output is under considerable modulatory influence from the periphery. This influence is of two main types: a tonic excitatory feedback onto the central generator; and phasic information to co-ordinate the rhythm. Wilson (1964, 1967) concluded that, in the flight system of locusts, peripheral proprioceptive feedback exerts a tonic excitatory effect on the output frequency and does not entrain the rhythm. It has since been shown that phasic input from the wing stretch receptors (produced by artificially driving one wing) can entrain the flight rhythm (Wendler, 1974). Burrows (1975) has shown that the wing stretch receptors connect monosynaptically with ipsilateral flight motoneurons and are capable of causing subthreshold waves of depolarisation in depressor motoneurons. Thus the stretch receptors can affect the time of spiking of motoneurons and influence the amplitude of the upstroke and the phase relationship between motoneuron spikes. A phasic influence of wingbeat synchronous feedback has also been demonstrated on the fibrillar and non-fibrillar flight muscles of flies (Heide, 1974). The role of limb and wing proprioceptors in flight, walking, song and courtship behaviour of insects, chelicerates and myriapods is extensively reviewed by Wright (1976). Entrainment of the pyloric rhythm of lobsters with a rhythmic synaptic input

is also possible (Ayers and Selverston, 1977). Tonic excitatory feedback is documented for hermit crab cheliped flexion behaviour (Field, 1976) and locust flight (Gewecke, 1977), and a tonic inhibitory feedback has been described in the cockroach walking system (Pearson, 1972).

In summary: Peripheral feedback has been shown to be capable of having a phasic co-ordinating function and a tonic excitatory or inhibitory function. In addition, resistance reflexes, whose prime role may be in controlling posture, can act to intensify power strokes in a similar way to excitatory reflexes described in the feeding rhythm of Helisoma trivolvis. This function is probably also carried out by tension afference. Positive and negative feedback reflexes have been described.

Regulation of Rhythmical Activity from Higher Centres

The study of the regulation of oscillators from higher centres is essentially the study of command neurons. A command neuron can be defined as a single identifiable cell which is capable of releasing organised segments of behaviour (Kennedy, 1969; Bowerman and Larimer, 1976). Most of the work on this topic has been with identifiable fibres dissected out of major nerve trunks. With the notable exception of the results obtained from the stimulation of single cells in Tritonia (Willows, 1967), most of the information characterising command elements has been obtained from crustacean systems. Bowerman and Larimer (1976) have recently reviewed the literature on command neurons in crustacea so a detailed consideration of the subject here would be redundant. What follows is a short summary of the well-established characteristics of command fibres.

1) The action pattern produced by stimulation of a command fibre is dependent on the frequency of stimulation. Also, there is usually a threshold frequency for activation (e.g. Davis and Kennedy, 1972).

2) Repeated stimulation leads to a loss of effectiveness. The mechanisms underlying this loss of effectiveness are not known (Bowerman and Larimer, 1976).

3) The number of command neurons affecting particular behaviours is extremely variable (e.g. 2 from the supraoesophageal ganglia affecting stomatogastric rhythmicity in spiny lobsters - Dando and Selverston, 1972; 20 inhibitors and 14 accelerators of cardiac activity in crayfish - Field and Larimer, 1975).

4) Commands to inhibit behaviour are well known (e.g. Dando and Selverston, 1972; Bowerman and Larimer, 1974; Field and Larimer, 1975). Inherent in the study of command is that of command-derived inhibition (Kennedy, 1975). This is a process whereby sensory neurons are presynaptically inhibited by a corollary discharge from command elements to prevent unnecessary sensory information (reafference) from disrupting the action pattern.

5) The motor patterns produced by command fibre stimulation are centrally generated and do not need peripheral feedback for their expression (Kennedy, Selverston and Remler, 1969).

Of equal importance to command neurons in the generation of intersegmental rhythms like locomotory rhythms, is a class of interneurons termed co-ordinating neurons (Stein, 1974). They transmit precise timing information about the state of one segmental oscillator to others. Intersegmental interneurons co-ordinate the heartbeat rhythm of leeches and a possibility exists that they act as endogenous oscillators timing the rhythm also (Thompson and Stent, 1976b,c). An important byproduct of this work is that it unequivocally demonstrates that leech segmental ganglia should be considered as identical only with extreme caution.

Gillette and Davis (1977) have recently shown that command neurons of the feeding rhythm of Pleurobranchaea (the Metacerebral giant cells) are

integral components of the network they drive. This is also true of the cardiac sac pacemaker (C.D.2) in the stomatogastric system of Palinurus vulgaris (Moulins and Vedel, 1977). The Metacerebral giant cells receive central feedback from the motoneurons of the network. To account for the functional redundancy implied by this arrangement, they propose that the command role of a neuron may derive from special access to the natural sensory input which drives the behaviour. This observation coupled with the fact that work on the nature and integration of the natural input to command neurons is limited indicates that studies on the initial and terminal control, via sensory input, of rhythmical behaviour may prove fruitful.

Advantages of the Preparation

The digestive tract of decapod crustaceans possesses a number of rhythmical activities: from the rhythmical biting actions of the mandibles (Wales, Macmillan and Laverack, 1976b) and the opening and closing of the mouth; through the oesophageal, cardiac sac, gastric mill, and pyloric rhythms (Spirito, 1975; Moulins and Vedel, 1977; Selverston, Russell, Miller and King, 1976); to the peristaltic movements of the hindgut and rhythmic anal contraction (Winlow and Laverack, 1972a,b,c; Muramoto, 1977). It has already been pointed out (see above) that there is a commonly held idea that those activities which occur in a variable environment tend to rely more heavily on peripheral feedback for modification and sensitivity than those that do not. In the crustacean decapod digestive tract examples of both extremes can be found. The movements of the mandibles during biting on objects which are extremely variable in size, texture and rigidity are continually modified by position (proprioceptive) and tension (load) sensitive afference (Macmillan, Wales and Laverack, 1976), whereas the pyloric filter encounters particles of almost uniform size, and the pyloric rhythm can be maintained in the absence of all afferent activity (Selverston, 1977). The other rhythms

mentioned are probably intermediate to these extremes.

Although most models of rhythmical behaviour make provision for a central oscillator, it is only in a very few cases that the nature of the oscillator has been demonstrated (see e.g. Kandel, 1976, Chapter 10). Notably this is true for the gastric and pyloric rhythm of lobsters (Selverston, 1977). In this instance both rhythms are generated in the stomatogastric ganglion. The gastric rhythm is a good example of one driven by a network connectivity oscillator, while the pyloric demonstrates the control exerted by endogenous oscillators. The neuronal mechanisms underlying the other rhythms are beginning to be elucidated (Moulins and Vedel, 1977). For obvious reasons all the rhythms of the intestinal tract must be co-ordinated with each other. A co-ordinating link between the foregut and hindgut is perhaps less important, but, ignoring hindgut and anal activity, six rhythms remain (mandibular, oral, oesophageal, cardiac sac, gastric, and pyloric). In this foregut system it has been demonstrated that a measure of control from command fibres exists (Dando and Selverston, 1972; Dando, Chanussot and Nagy, 1974; Hermann and Dando, 1977), and that interganglionic neurons play a co-ordinating role (Russell, 1976; Vedel and Moulins, 1977). A more detailed review of the neurophysiology of the foregut of decapod crustaceans will appear in the introduction to Chapter 4.

It is evident from the above that the decapod crustacean foregut provides an ideal preparation for many studies concerned with the neuronal control of rhythmic behaviour. There is a profusion of rhythmic activities which rely on sensory feedback to different extents; are co-ordinated with each other; and are under the influence of higher nervous control. Apart from this, invertebrates, and arthropods in particular, have many advantages for the experimental neurobiologist. Their behaviour patterns vary in complexity

while the neuromuscular and sensory systems underlying these behaviours are readily accessible, long-lived and have a great deal of similarity to other (vertebrate) systems. Furthermore the individual muscular and nervous elements are relatively large permitting microelectrode analysis of synaptic events; there is a sparseness of efferent innervation which can easily be characterized; and there is already a wealth of knowledge about invertebrate neuromuscular mechanisms (Atwood, 1967; Kennedy, Selverston and Remler, 1969; Sherman, Fourtner and Drewes, 1976).

Objects of Research and Plan of Thesis

1) When the work for this thesis was started there was a considerable gap in the knowledge of the mechanisms controlling the passage of food in the foregut of the lobster, Homarus gammarus. Little was known of the control of the rhythmical movements of the mouth and the oesophagus. The project was initiated to describe this activity and make an exploratory study of its control. As the knowledge of the area and the literature on the subject increased, the primary aim of the work became to elucidate the feedback effects of two bilateral chemosensory organs present at the junction between the oesophagus and the cardiac sac, and to determine their role in the control of food ingestion in Homarus gammarus. It was hoped that this might yield information of a more general kind concerning the role of chemosensory afference in behaviour.

2) Chapter 2 describes the gross neuromuscular anatomy of the labrum and oesophagus, and the more detailed structure of the sense organs at the oesophageal/cardiac sac valve. This provided the necessary basic knowledge for investigations of the physiology of these structures.

3) Chapter 3 describes the rhythmical movements which the labrum (the upper lip or anterior rim of the mouth) undergoes during the chewing

and swallowing of food. Such a description is a prerequisite for future studies on its neuronal control and its co-ordination with the documented mandibular and oesophageal rhythms.

4) Chapter 4 reports on experiments designed to discover the role of the oesophageal sensors in the control of oesophageal peristalsis. Also the responses of labral mechanoreceptors and the neuronal burst pattern controlling oesophageal peristalsis are briefly described.

5) In the final chapter the more general significance of the results is discussed and suggestions are made where further research in the area might be profitable.

CHAPTER 2

LABRAL AND OESOPHAGEAL ANATOMY

LABRAL AND OESOPHAGEAL ANATOMY

1. INTRODUCTION

A recent paper by Maynard and Dando (1974) has done a lot to clarify our present knowledge of the stomatogastric neuromuscular system of decapod crustaceans. This has been amplified by Meiss and Norman (1977a,b). These papers, however, restrict themselves to descriptions of the cardiac sac, pylorus and gastric mill. To obtain any information about the structure and musculature of the labrum and oesophagus of these animals one must delve further back into the literature.

Review of Labral Anatomy

The labrum is a small structure (4-6mm in length) and perhaps for this reason it has largely been ignored or mentioned merely in passing. Huxley's "The crayfish: an introduction to the study of zoology" (1880) is still the standard anatomical text for macruran decapods. In this work the labrum is dismissed as a wide shield-shaped plate which overlaps the mouth, and which is strengthened by three pairs of calcifications in a longitudinal series. For Eupagurus (Jackson, 1913), Nephrops (Yonge, 1924) and Palaemon (Patwardhan, 1937) the description is no better. In Cancer ... "the labrum is a soft fleshy lobe attached to the middle region of the posterior border of the epistoma. It is surrounded near the middle by a calcareous ring which gives off a median posterior prolongation. At each side of this median plate is a soft fold." (Pearson, 1908).

Paterson (1968) states that, for Jasus lalandi, "... the labrum is furnished with a complicated musculature, comprising what appear to be constrictor, levator, abductor and adductor muscles...", and Fryer (1977) describes the labrum of Atyid prawns as being "... provided with muscles running largely fore and aft...". However, the only attempts to describe

the labral musculature in detail have been by Lemoine (1868), Mocquard (1883) and Ringel (1924). Lemoine describes five muscle bundles in the lobster's labrum without naming them. The studies of Mocquard and Ringel were with the crayfish and give essentially the same information. They describe four paired muscles: 1) internal labral retractor; 2) external labral retractor; 3) median labral retractor; 4) labral levator; and one intrinsic transverse muscle of the labrum.

Knowledge of the innervation of the labrum is almost totally lacking. The only precise information available is that two paired nerves pass from the oesophageal nervous system to innervate the labrum: 1) the outer labral nerve; and 2) the inner labral nerve. (Most authors, but see e.g. Chaudonneret, 1956). Also, Dando (1969) has briefly mentioned, but not described, large and small bipolar sensory cells present in the labrum of Homarus.

To summarise, at present it is known that the labrum of decapod crustaceans is: 1) a shield-shaped lobe overhanging the mouth and strengthened by calcareous thickenings;

2) invested with a complex musculature (with five named muscles);

3) innervated by two paired nerves and some sense cells.

Review of Oesophageal Anatomy

In all described reptantian decapods the oesophagus is known as a short, chitin-lined tube which connects the mouth and mandibles to the cardiac sac. However, its position of entry into the cardiac sac can vary within the group. For example, in Callinectes (Brachyura) the oesophagus enters the anterior ventral border of the cardium; in Homarus (Macrura) its position is more ventral; and in Panulirus (Palinura) it is almost vertical and enters towards the posterior end of the stomach. (Maynard and Dando, 1974).

These differences probably only reflect the differences in the relative positions of the mouth and stomach as a result of the variation in the overall form of each animal. The oesophageal walls are often thrown into longitudinal ridges. The most prominent of these is an antero-dorsal fold which is continuous with the labrum (Barker and Gibson, 1977). Between the oesophagus and cardiac sac is a simple valve formed from the invagination of their walls. There can be variation in the number and position of the lobes comprising this valve. Jasus has one ventral and two lateral lobes (Paterson, 1968). Homarus has been described as having lobes encircling the orifice except anteriorly (Bocquard, 1883) and as having a trilobed valve with one dorsal and two lateral portions (Barker and Gibson, 1977).

The oesophageal musculature typically consists of upper and lower oesophageal dilators, lateral oesophageal dilators, posterior oesophageal dilators and a complex oesophageal constrictor with longitudinal and circular fibres (Bocquard, 1883; Paterson, 1968; Pearson, 1908; Yonge, 1924). Bocquard (1883) also describes an oesophageal elevator and Maynard and Dando (1974) include in their Figure 8 (a lateral view of the stomach muscles of Homarus americanus) a narrow muscle which has two insertions on the lateral oesophageal wall at the level of the oesophageal/cardiac sac valve. There has been no attempt to describe the form of the oesophageal constrictor.

The main nerves of the oesophageal system have been well described by a number of authors (for a review see Bullock and Horridge, 1965) and a brief description of the general layout will appear in the Results section of this chapter. However in all cases there is very little detail about the courses of the finer branches, innervating specific oesophageal muscles.

Dando and Maynard (1974) have provided an excellent review of the sensory innervation of the foregut of decapod crustaceans primarily based on the early work of Allen (1894), Bingel (1924) and Orlov (1926a & b) and on

their own observations with Panulirus argus. Of the six main groups of receptors they describe, two are germane to this thesis:

1) Receptors monitoring movements of the lower oesophagus and mouth;

2) Probable chemoreceptors on the oesophagus and lower cardiac sac.

Group 1 consists of the "mouth part receptors", MPR1, 2 and 3 (Laverack and Dando, 1968). These are innervated elastic strands around the base of the oesophagus. The name "mouthpart receptors" has been questioned (Wales, Macmillan and Laverack, 1976a). It is suggested that as they respond to labral, mandibular, paragnathal and oesophageal movement their present name is misleading. It is further suggested that they be renamed peri-oesophageal receptors. This may be necessary, but to avoid confusion any reference to these receptors in this thesis will be as MPR1, MPR2, or MPR3. The anatomy and physiology of these organs have been extensively studied in Homarus vulgaris (synonymous with H. gammarus) (Laverack and Dando, 1968), Nephrops norvegicus, Astacus leptodactylus and Panulirus argus (Moulins, 1969; Moulins, Dando and Laverack, 1970).

Group 2 can be subdivided into:

a) Innervated hairbands projecting into the ventral canal of the cardiac stomach (Ringel, 1924). Dando and Maynard (1974) could not confirm the presence of these neurons with Methylene blue staining. The position of these presumptive chemoreceptors in the cardiac sac places them outside the context of this thesis.

b) Two bilaterally symmetrical groups of neurons were first described in Homarus by Allen (1894). Orlov (1926a) redescribed them in Astacus leptodatylus and called them "Geschmacksorganen" which implies that

they function as organs of taste. Their peripheral processes pass through the oesophageal cuticle but are not associated with any hairs. Each organ is innervated by a dorsal branch of the ipsilateral superior oesophageal nerve. Dando and Maynard (1974) note the similarity of the structure of these organs in Astacus with a presumptive chemoreceptor on the hypopharynx of the cockroach Blabera (Moulins, 1968). They also note that the arrangement of the organ in Panulirus argus is different, there being no concentration of the cells into distinct groups. In this work these organs will be referred to as the anterior oesophageal sensors (A.O.S.).

c) Innervated pegs in a series of sensory plates on the surface of the ventral cardiac gutter (Ringel, 1924). The pegs project through the cuticle and sit in small depressions. Dando and Maynard (1974) found them in Callinectes and Panulirus where the innervating nerve is the ventral cardiac branch of the postero-lateral nerve. In Homarus (this thesis) similar structures can be found although they occur on the posterior wall of the oesophagus at the level of the oesophageal/cardiac sac valve. They are innervated by the ventral-posterior oesophageal nerve and will be referred to as the posterior oesophageal sensors (P.O.S.).

Objects of Research

1. The above short review of the literature on the anatomy of the labrum reveals that very little is known about its structure, musculature and innervation. The first object of this anatomical study is therefore to provide an adequate knowledge of labral anatomy so that subsequent investigations of its role in feeding might be rendered more meaningful.

2. The oesophageal anatomy is better represented in the literature. However, preliminary observations on Homarus showed that discrepancies exist between its anatomy and that reported for other decapod crustaceans. This

is particularly noticeable for the muscles at the oesophageal/cardiac sac valve, and it seemed useful to treat this as a separate entity with its own musculature. Because of this and because of the recent interest in the control of oesophageal peristalsis in decapod crustaceans (Spirito, 1975; Selverston, Russell, Miller and King, 1976) the second aim of this section is to give an up to date description of the oesophageal neuromuscular system. This may lay the groundwork for investigations into the way in which peristalsis is effected.

3. Four presumptive chemoreceptors have been described on the oesophagus of decapod crustaceans (2 A.O.S. and 2 P.O.S.). In Homarus these are present in a ring around the oesophageal/cardiac sac valve and are the most obvious sense organs present. Very little is known about their anatomy save that they are present. The final aim of this section is to elucidate their structure, the better to understand their function.

In summary: this anatomical study was initiated to describe the foregut of Homarus gammarus from the labrum to the oesophageal/cardiac sac valve paying particular attention to these structures.

2. MATERIALS AND METHODS

Lobsters, Homarus gammarus, were provided by the Gatty Marine Laboratory, and maintained in large tanks of circulating, aerated sea water. They were fed twice a week, except when, for the purposes of a particular experiment (see Chapter 3), it was desirable to use animals which had been starved for a short while.

The morphology and gross muscular anatomy of the labral/oesophageal complex were examined by the dissection of fresh specimens in sea-water. In some instances, the soft tissues of the labrum were dissolved away with

concentrated sodium hydroxide (NaOH) so that the details of the skeletal anatomy could be obtained. The anatomy of the nervous system of this area, including the commissural ganglia, oesophageal ganglion, major nerve trunks and peripheral sensory systems, was examined using the vital stain Methylene blue (Me. blue). A stock solution of 2% Me. blue in distilled water was added to the dissection dish in sufficient quantity to colour the sea water light blue. (Approximately 15 drops of stock solution/100mls sea water). Staining and further dissection were alternated until maximal staining occurred.

The length of time taken to stain depends on three main factors:

(a) the final concentration of Me. blue in the bath;

(b) the temperature of the bath;

(c) the proximity of the tissue to the surface of the staining solution. (For a review of Me. blue staining technique, see W. Wales, 1972).

Sufficiently stained preparations were processed and mounted as permanent preparations. The tissue was fixed for several hours, usually overnight (exact timing is not important, as long as fixation occurs for longer than 3 hours, and not more than 24 hours as the stain tends to leach out in the fixative) in a 10% solution of Ammonium molybdate in distilled water, dehydrated in three changes of absolute alcohol, cleared in Xylene, and mounted in polystyrene. The resulting preparations were examined on a Zeiss microscope using both normal and Nomarski-interference-contrast illumination, and photographed with an EXA1 camera (Ihagee, Dresden) using Ilford Pan F film.

Scanning Electron Microscopy

Tissue was prepared for the scanning electron microscope (S.E.M.) in the following way. The appropriate piece was removed from an animal and pinned, with the relevant surface uppermost, in a wax bottomed Petri dish

containing 4% Formalin in sea water. After fixation, the tissue was washed in distilled water, dehydrated in an Acetone series and critical point dried with CO2. It was then coated with gold palladium and viewed on a Cambridge S600 Stereoscan. Photographs were taken with an EXA1a camera (Ihagee, Dresden) using Ilford FP4 film.

Histology

Selected pieces of tissue were excised and fixed in sea-water Bouins. The tissue was then embedded in paraffin wax, and 10µm serial sections were cut. The sections were cut out at 10µm to facilitate counting of the individual endings. They were stained with either Mallory's triple stain or Heidenhain's Azan stain (Pantin, 1964), and mounted in Euparal. Sections were viewed on a Leitz ortholux microscope and photographed with a Leica camera (Leitz) using Ilford Pan P. film.

3. RESULTS

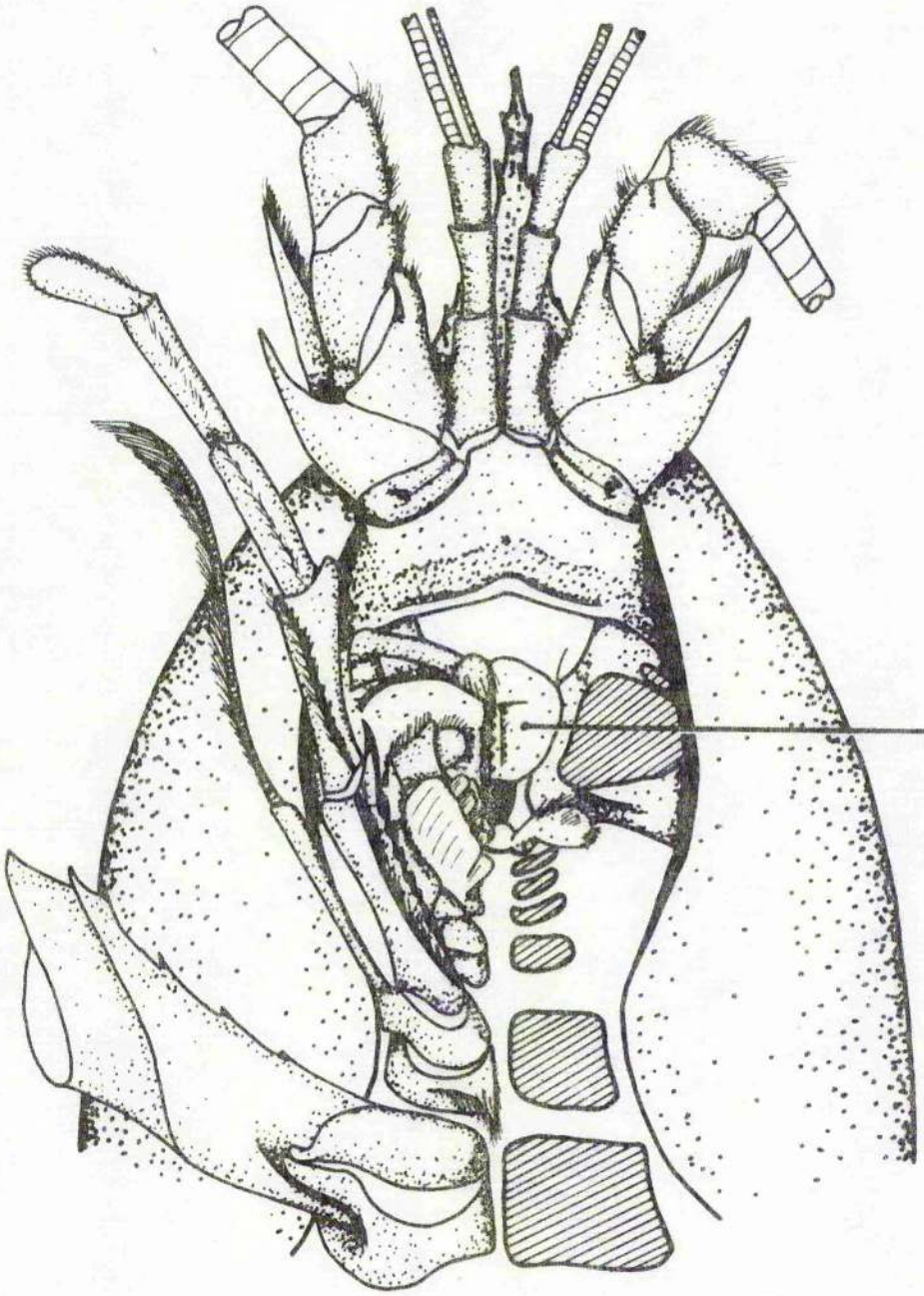
A. LABRUM

External Morphology

The labrum is the upper lip (Fig. 1), and, at rest, its edges lie between the molar and incisor processes of the mandibles. It can best be described as an out-pouching of the anterior border of the mouth, with arthrodial membrane attaching it proximally to the epistoma, mandibles and oesophagus. These attachments form an almost circular opening into the lumen of the labrum. The structure is bilaterally symmetrical and resembles a slipper, with a large ventral sclerite (the sole), and a dorsal, posterior lobe (the toe). This sclerite determines the shape of the structure, and it is roughly triangular with a broad anterior border narrowing to a blunt point posteriorly. In average sized lobsters (cephalothorax length - 10cm from tip of rostrum to end of thoracic carapace) the labrum is about 4mm long, and 3mm wide at its broadest point.

Figure 1

Antero-ventral view of head region of a lobster to show position of labrum. The mouth appendages and chela of the left side have been omitted to clarify the diagram.


LABRU

Skeleton

The skeleton of the labrum is extremely flexible. However, although the skeletal parts can be deformed in any plane, their elasticity maintains the overall shape of the labrum at rest.

The most conspicuous supporting structure in the labrum is the ventral scutiform sclerite (Fig. 2). It has 3 faces, an anterior face, and two lateral ones. These are angled against each other to form a shallow triangular dish. The lines delineating the three faces run posteriorly and antero-laterally (left and right) from approximately the midpoint of the sclerite. The median line is marked by a ridge, but the other two are less distinct. A wide apron of arthrodial membrane joins the anterior edge of the scutiform sclerite to the supra-labral ridge of the epistoma.

At the junction between the oesophageal and lobar cuticle can be found the furcular sclerite. This is composed of two curved struts which run dorsally, posteriorly and medially from their anterior attachments with the lateral edges of the scutiform sclerite (Fig. 3). These joints are located at notches between the edge of the anterior face and the edges of the lateral faces. Interposed between the anterior end of a strut and the scutiform sclerite is a small piece of thickened cuticle, the nodular sclerite, which lends greater flexibility to the joint. To form the furcular sclerite, the posterior ends of the two struts are fused together in the midline of the anterior oesophageal wall, and there is a small projection running dorsally from the point of fusion in the oesophageal cuticle. The furcular sclerite thus forms a flexible bow-shaped arch into the lumen of the labrum.

On either side of the scutiform sclerite are two hook-like thickenings of the cuticle. These are the falciform sclerites (Fig. 3). Their broad ventral ends abut the lateral edge of the scutiform sclerite's anterior face just anterior to the anterior articulations of the furcular

sclerite. They narrow dorsally and curve laterally over the medial internal rims of the mandibles.

The apical sclerite is a slightly curved, triangular thickening of the lobar cuticle just posterior to the scutiform sclerite. The sides of the triangle are demarcated by three ridges, one transverse, and two longitudinal. The latter do not meet at the apex of the triangle, but terminate before that point.

In addition to the structures described above, there are two more areas of cuticular thickening on each side. These, in contrast, are merely the sites of muscle insertions, and play no part in maintaining the morphological integrity of the labrum. They are bilaterally situated on the apron of cuticle which joins the labrum with the epistoma. They are not easily distinguishable from the rest of the cuticular membrane, and serve as the insertions of muscle L6 (see below).

Musculature

The movements and shape of the labrum are controlled by 5 pairs of extrinsic muscles, one solitary and 4 pairs of intrinsic muscles. All of these muscles are bilaterally symmetrical. Because of the difficulty in relating the physiological actions of muscles to predictions of their actions based on anatomical evidence, the current convention is to number muscles, as opposed to giving them descriptive names. This convention will be observed with respect to the labral musculature, and the muscles are numbered starting with the most medial, and proceeding laterally. The numbering of the cardiac sac muscles follows Maynard and Dando (1974). The origins, insertions, size and shape of the muscles will be described, with predictions of their possible actions. Figures 4 and 5 depict the labral musculature from a lateral aspect, and Figures 6 and 7 show it from a dorsal aspect. They should be referred to throughout the following description.

Figure 2

Labral skeleton. Ventral aspect

A.P. - anterior face

Ins.L6 - cuticular thickenings at insertions of muscle L6

L.P. - lateral face

Sc. - sclerite

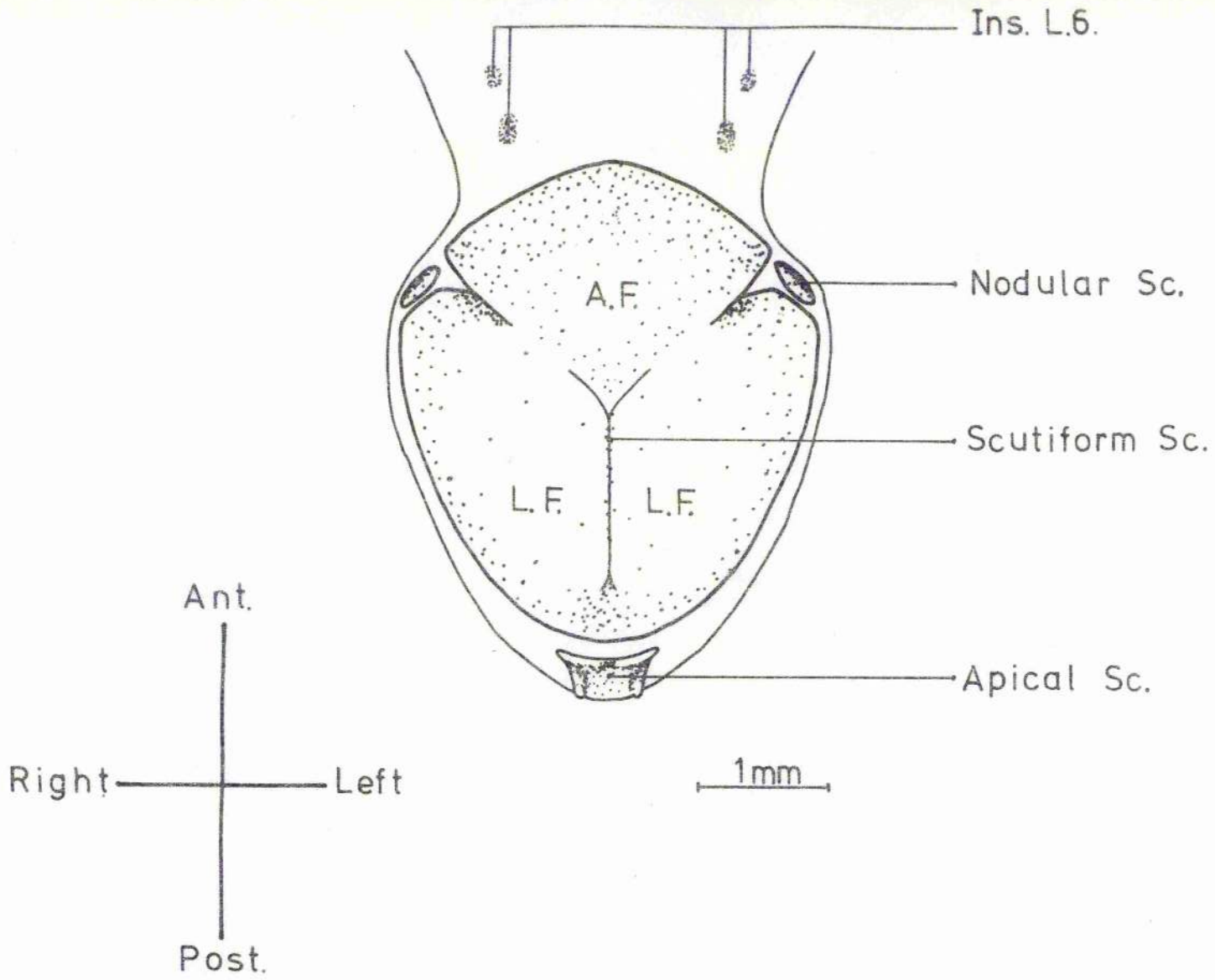

Ins. L.6.
A.F.
Nodular Sc.
Scutiform Sc.
L.F.
L.F.
Apical Sc.
Ant.
Right
Left
Post.
1mm

Figure 3

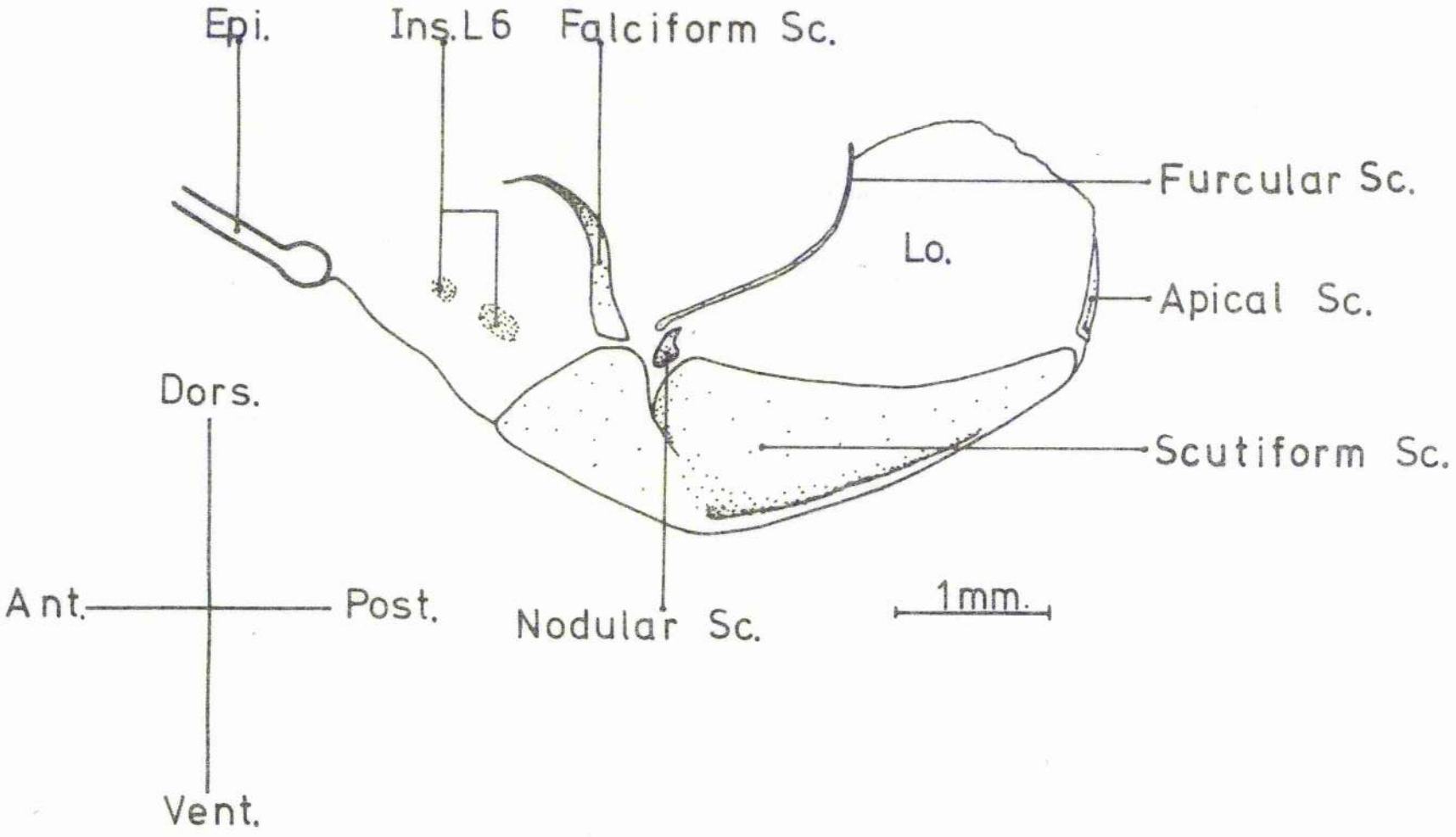
Labral skeleton. Left lateral aspect

Epi. - Epistoma

Ins.L6 - cuticular thickenings at the insertions of muscle L6

Lo. - lobe of labrum

Sc. - sclerite


Epi.
Ins.L6
Falciform Sc.
Furcular Sc.
Lo.
Apical Sc.
Dors.
Scutiform Sc.
Ant.
Post.
Nodular Sc.
1mm.
Vent.

(a) Intrinsic

L1 .... is the only labral muscle which is not paired, although it is symmetrical about the longitudinal midline of the labrum. It is a broad transverse muscle with insertions on the antero-lateral borders of the scutiform sclerite's lateral faces, just posterior to their joints with the nodular sclerites. The action of this muscle will be to constrict the labrum laterally.

L2 .... originates on the scutiform sclerite just lateral to its longitudinal midline and between the anterior and lateral faces. It runs dorso-posteriorly, passing ventral to L1 and inserts on the lobar cuticle just posterior to the furcular sclerite. Contraction of this muscle will tend to protract the labrum, and retract its lobe.

L3 .... runs posteriorly from its origin on the anterior face of the scutiform sclerite (anterior and lateral to the origin of L2) to its insertion on the lateral edge of the apical sclerite. This muscle could shorten the labrum by flexing it about a transverse axis, and by retracting the lobe.

L7 .... is a broad muscle which has its origin on the furcular sclerite and passes ventrally to insert in the middle of the lateral face of the scutiform sclerite. This muscle will constrict the labrum dorso-ventrally. However, assuming that the furcular sclerite will move ventrally only slightly, due to its attachment to the oesophageal cuticle, it may be more accurate to describe it as levating the labrum.

L6 .... has its origin on the anterior edge of the scutiform sclerite's lateral face, medial to the insertion of L1. It runs posteriorly, passing ventral to L1 and lateral to L7. It has a diffuse insertion in the toe of the labrum with two main bundles. L6a inserts on the posterior lateral edge of the scutiform sclerite, and L6b inserts on the lateral edge of the

apical sclerite. If L8 of the right and left sides of the labrum were to contract in concert, they would, similar to L3, shorten the labrum and retract the lobe. However, due to the separation of their origins, contraction of L8 on only one side will tend to bend the labrum towards that side.

(b) Extrinsic

L4 .... is the largest muscle in the labrum and its contraction will undoubtedly retract the labrum. Its origin is on a large cuticular peg, the median apodeme of the supra-labral ridge of the epistoma. It passes posteriorly from this, ventral to L1, and has a large, fairly diffuse, insertion on the lateral face of the scutiform sclerite, to one side of the longitudinal midline.

L5 .... has its origin on the same cuticular peg as L4. It runs dorsal to L4, passing ventrally to L1, but continues past the insertion of L4 to insert on the apical sclerite. This muscle probably acts to shorten the lobe.

L6 .... travels ventrally from a very broad diffuse origin on the lateral anterior oesophageal wall, and splits into three main bundles. L6a, the smallest, runs anteriorly and inserts on a small area of cuticular thickening in the apron of arthrodial membrane which connects the labrum to the supra-labral ridge. L6b inserts posterior to L6a in the same fashion. L6c is a largish bundle which passes almost directly ventrally from the origin and inserts on the lateral corners of the anterior face of the scutiform sclerite. Contraction of L6 will tend to rotate the scutiform sclerite about a transverse axis passing through the nodular sclerites. If it contracts at the same time as L4 this will ensure complete retraction of the labrum by bending the scutiform sclerite.

O1a .... is a branch of the lower anterior oesophageal dilator (O1, see below). From its origin on the median apodeme of the supra-labral ridge O1 runs posteriorly, passes underneath L6, and has a large insertion mingling with the origin of L6. O1a branches off the main muscle, passes into the anterior fold of the oesophagus and inserts medially on the furcular sclerite. O1 will dilate the lower portion of the oesophagus and O1a will help in opening the mouth by pulling out the anterior oesophageal fold and retracting the labrum.

O4a .... is a branch of the oesophageal constrictor (O4). It passes over the posterior end of the O1a to insert medially along the furcular sclerite. Contraction will levate and protract the labrum.

Apart from L8, the predicted actions of the muscles have been described as if both muscles of a bilateral pair were contracting together. This may not be the case, and there is immense scope for fine alterations of labral movements and shape by differential contraction of the individual muscles of a pair. The range of movement and shape is further enlarged when one considers that some muscles will have different effects depending on which other muscles are active at the same time.

Innervation

The innervation of the right side of the labrum is shown from a medial aspect in Figures 8 and 9. Figure 10 depicts the total innervation from a dorsal aspect. Two paired nerves comprise the total innervation. These are the inner labral nerves (i.l.n.) and the outer labral nerves (o.l.n.). Further details on the relationship of these nerves with the oesophageal nervous system may be obtained from the section concerned with the oesophageal innervation.

(a) Inner Labral Nerve

This originates from the inferior oesophageal nerve (i.o.n.)

Figure 4

Labral musculature. Left lateral aspect

C.. - cardiac sac muscle

Epi. - Epistoma

L.. - labral muscle

Lo. - lobe

Mand. - mandible

O.. - oesophageal muscle

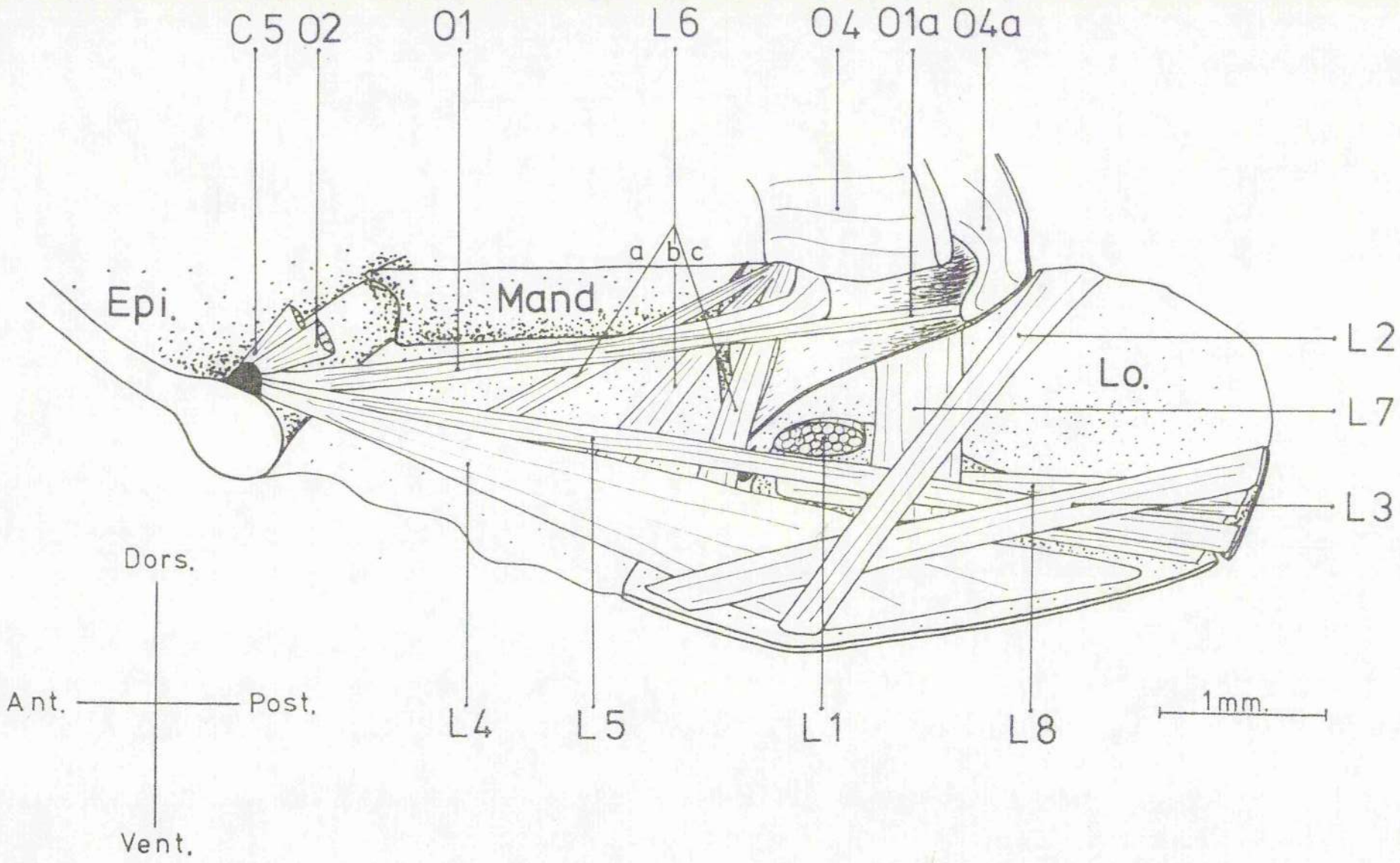

C 5
O2
O1
L6
O4
O1a
O4a
a
b
c
Epi.
Mand.
Lo.
L2
L7
L3
Dors.
Ant.
Post.
Vent.
L4
L5
L1
L8
1mm.

Figure 5

Labral musculature. Left lateral aspect. L2, 3, 4 and 5 removed.

Abbreviations as before.

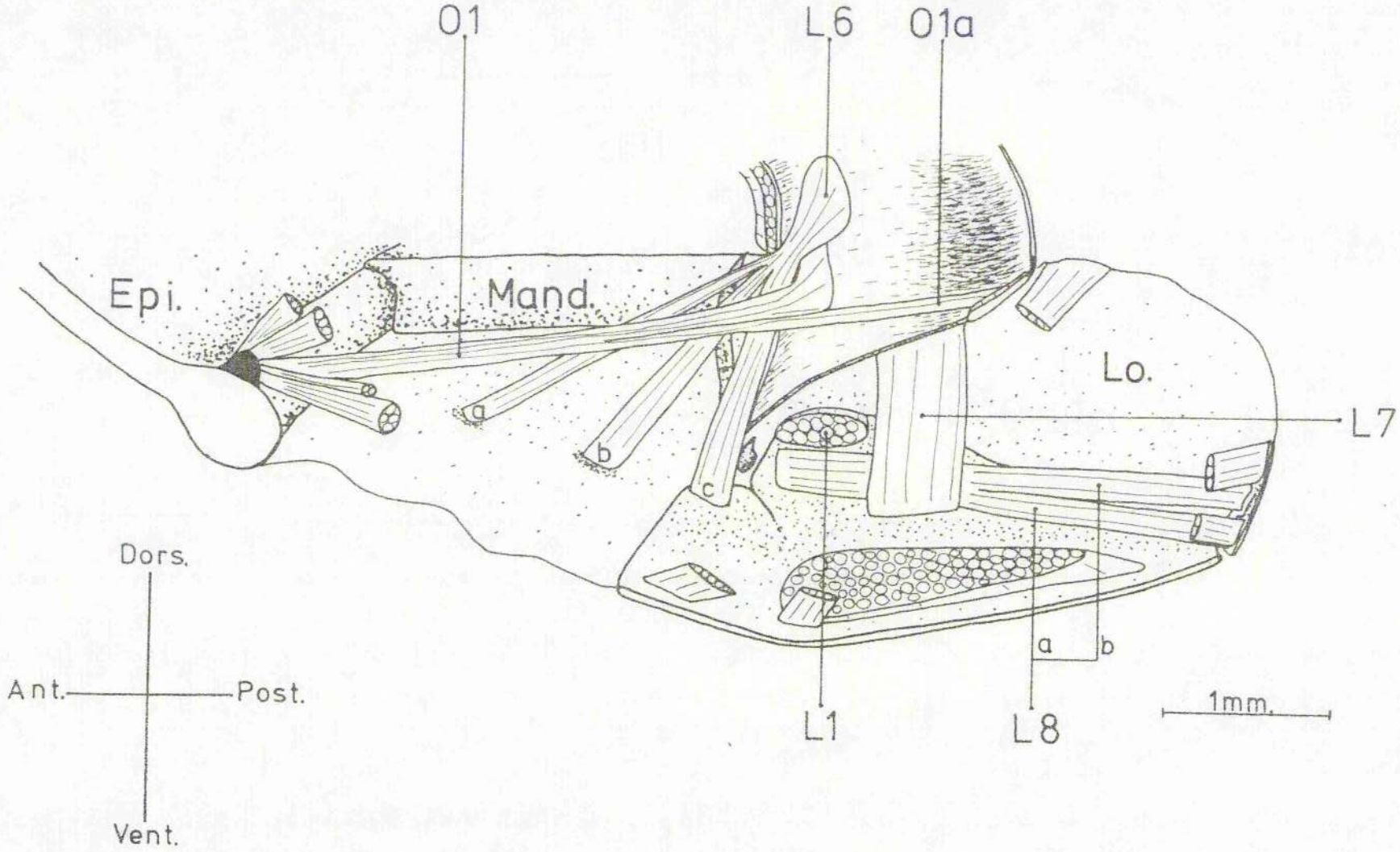

O1
L6
O1a
Epi.
Mand.
Lo.
L7
a
b
c
Dors.
Ant.
Post.
Vent.
L1
L8
a
b
1mm.

Figure 6

Labral musculature. Antero-dorsal aspect.

Fal. Sc. - Falciform sclerite

Pur. Sc. - Furcular sclerite

L.. - Labral muscle

Mand. - Mandible

O.. - Oesophageal muscle

S.L.R. - Supra-labral ridge

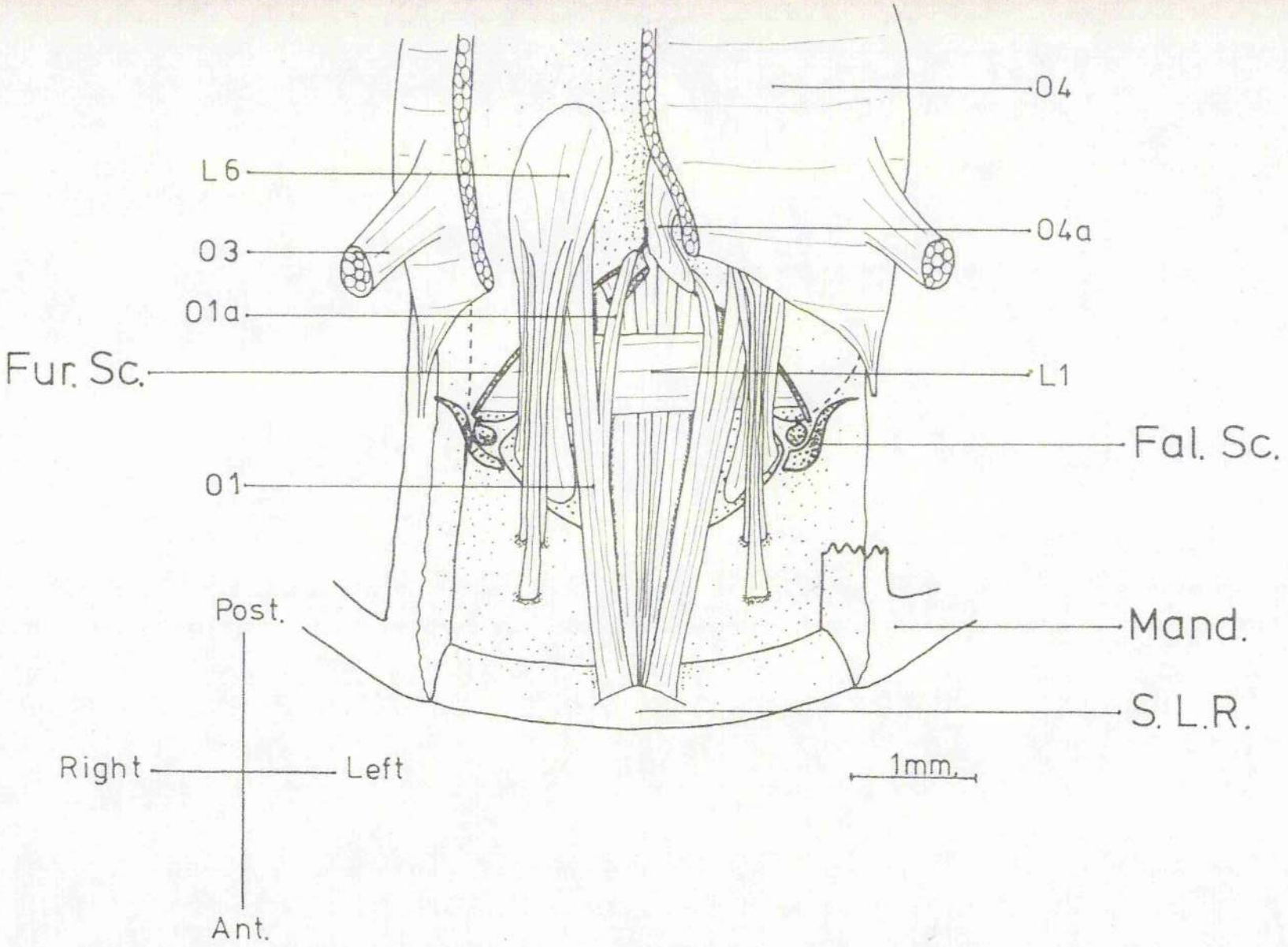
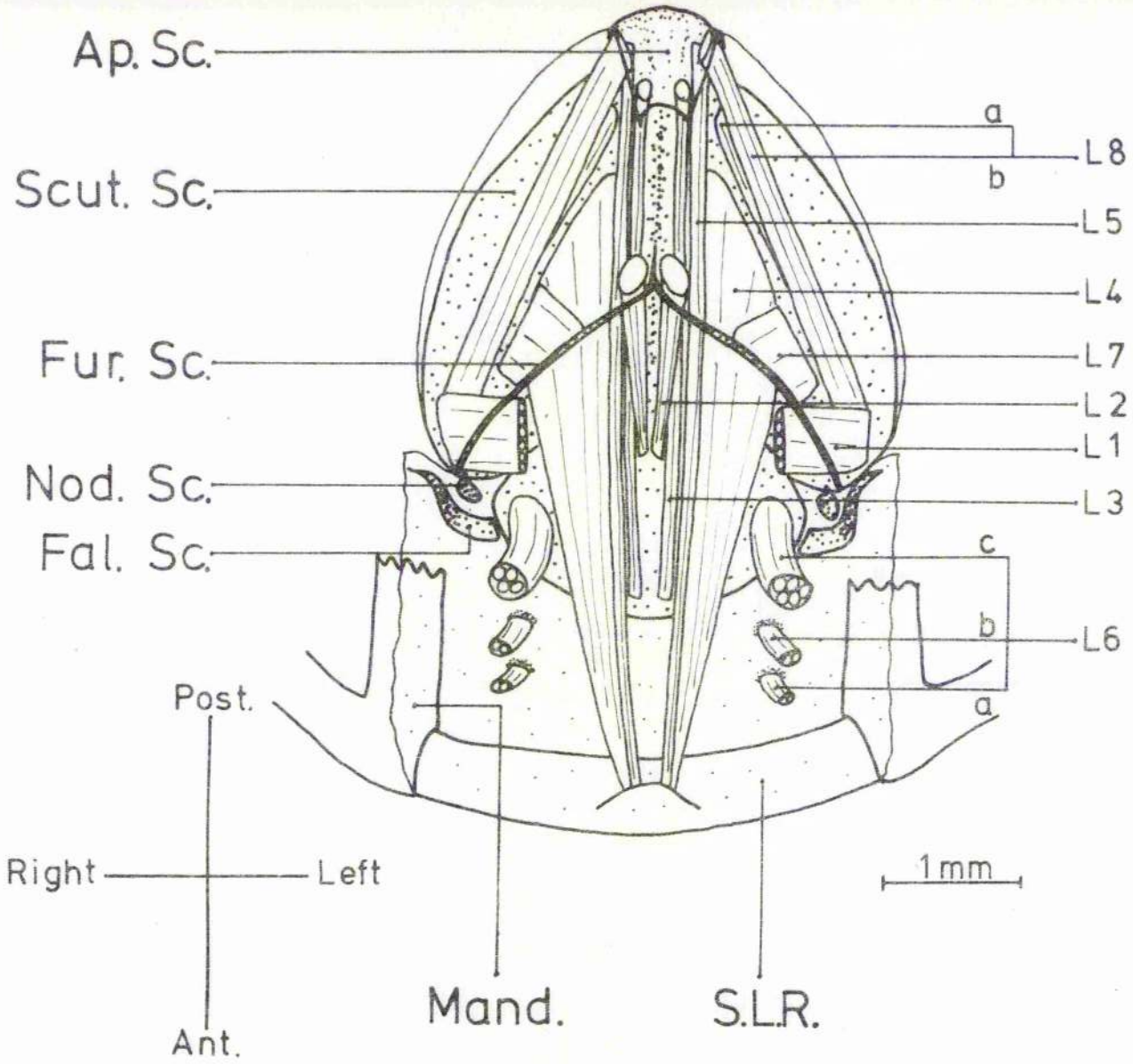

04
L6
04a
03
01a
Fur. Sc.
L1
Fal. Sc.
01
Post.
Mand.
S. L.R.
Right
Left
1mm.
Ant.

Figure 7

Labral musculature. Dorsal aspect. Oesophagus and oesophageal muscles removed, L1 cut.

Ap. Sc.	-	Apical sclerite
Pal. Sc.	-	Falciform sclerite
Pur. Sc.	-	Furcular sclerite
L1-8	-	Labral muscles
Mand.	-	Mandible
Nod. Sc.	-	Nodular sclerite
Scut. Sc.	-	Scutiform sclerite
S.L.R.	-	Supra-labral ridge


Ap. Sc.
Scut. Sc.
Fur. Sc.
Nod. Sc.
Fal. Sc.
a
b
L8
L5
L4
L7
L2
L1
L3
c
b
L6
a
Post.
Right
Left
Ant.
Mand.
S.L.R.
1mm

and carries fibres from the commissural ganglion and a few from the oesophageal ganglion area. It travels ventrally from i.c.n. over the surface of the oesophagus, to reach the dorsal surface of O1. Here it sends off branches to innervate O1 and L6 before proceeding into the labrum on the medial side of O1. When it reaches the level of L1 (the labral constrictor) it bifurcates, producing two branches. These will be designated i.l.n.(M), the medial root, and i.l.n.(L), the lateral root of i.l.n.

i.l.n.(M) - carries most of the motor axons innervating the labral musculature. Just after the bifurcation it branches to innervate L5 and L1. L1 is innervated by corresponding nerves from the right and left sides and some fusion between the branches from different sides may occur in the midline. The i.l.n.(M) proceeds posteriorly, medial to L5 and L4, and ventral to L1. It appears to innervate each of L2, L3, L4 and L5, twice with proximal branches serving the anterior ends, and distal branches serving the posterior ends of the muscles.

i.l.n.(L) - carries the sensory information from the labrum. From the bifurcation it runs posteriorly under L1 and lateral to L4 and L5. As well as serving three groups of sensory cells which will be described later, it innervates L7 and the dorsal and ventral bundles of L8.

(b) Outer Labral Nerve

This is purely a sensory nerve and it originates either directly out of the commissural ganglion or as a ventral branch of i.c.n. It runs antero-medially from its origin towards the labrum. On the way it gives off a small group of sensory cells (4-5). These innervate a discrete strand of tissue on the mandibular rim and the organ has been termed MFR1 (Dando and Laverack, 1968; Laverack and Dando, 1968). Anterior to this the o.l.n. enters the lumen of the labrum and becomes a tegumental nerve

Figure 8

Labral innervation. Medial aspect of the right side of the labrum in sagittal section to show the i.l.n.(M). The arrow indicates where the i.l.n.(L) has been cut to clarify the diagram.

i.l.n. - inner labral nerve

o.l.n. - outer labral nerve

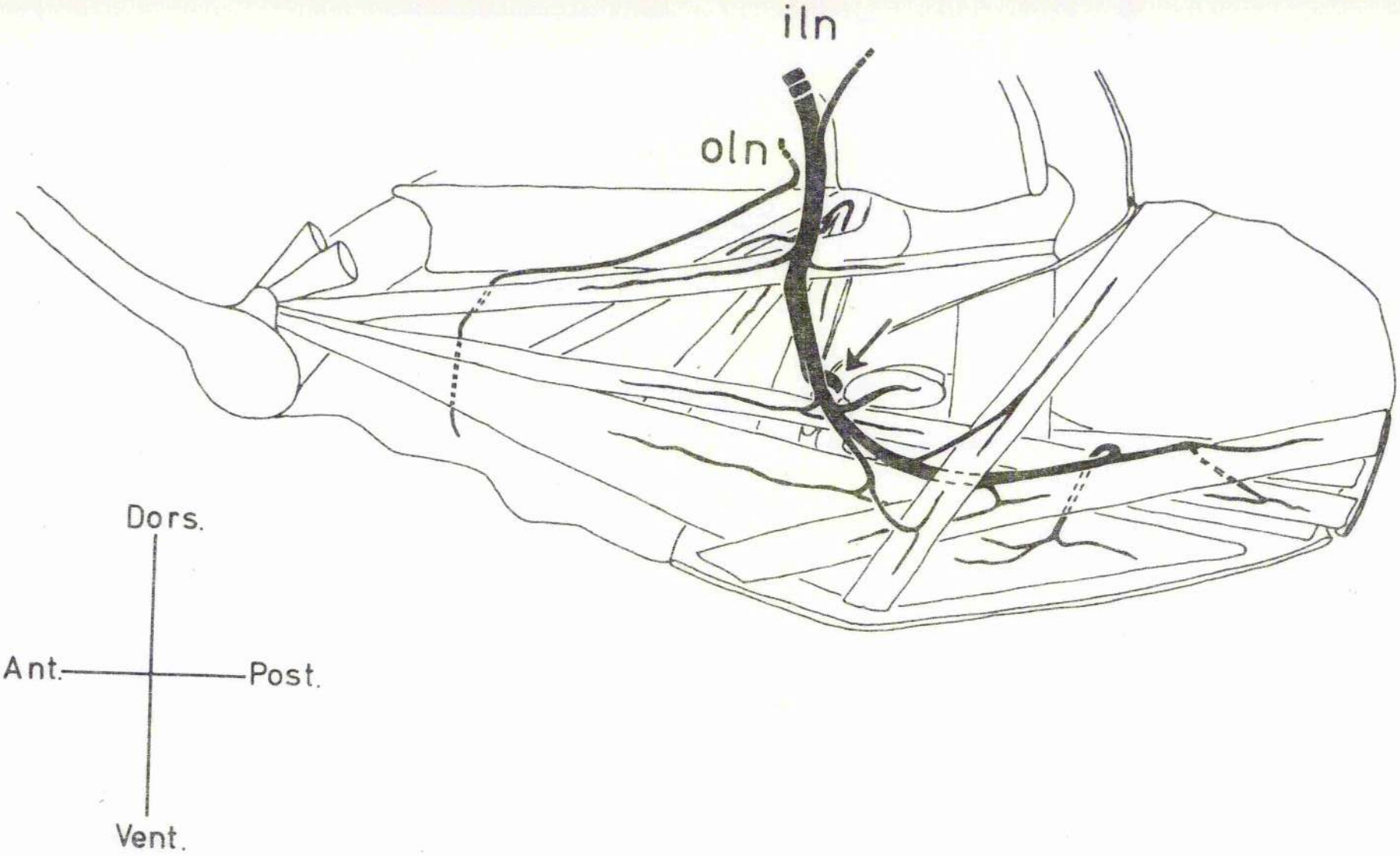
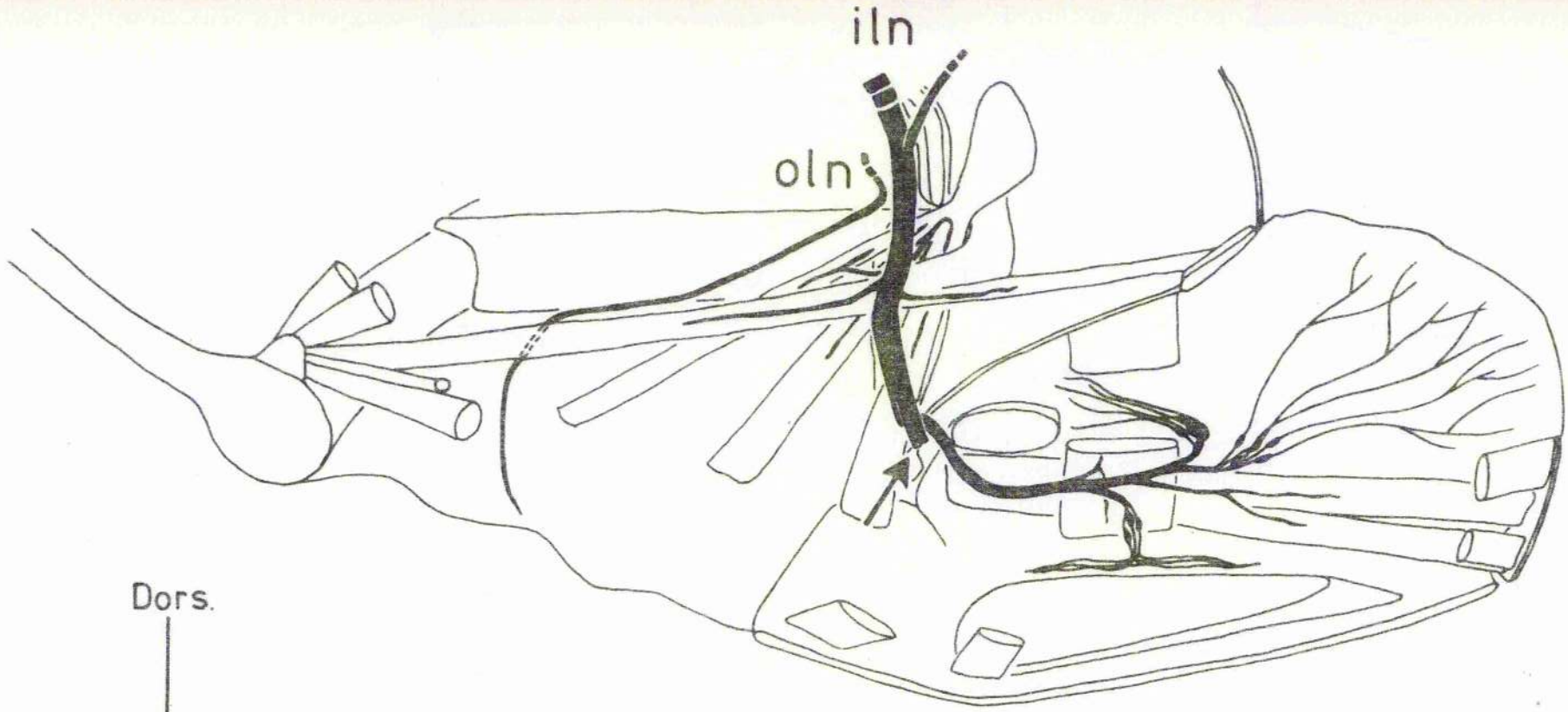

iln
oln
Dors.
Ant.
Post.
Vent.

Figure 9

Labral innervation. Medial aspect of the right side of the labrum in sagittal section to show the i.l.n. (L). The arrow indicates where the i.l.n. (M) has been cut to clarify the diagram. L2, L3, L4 and L5 have been removed, and L7 has been cut to show the peripheral processes of a group of nerve cells passing lateral to it.

i.l.n. - inner labral nerve

o.l.n. - outer labral nerve


iln
oln

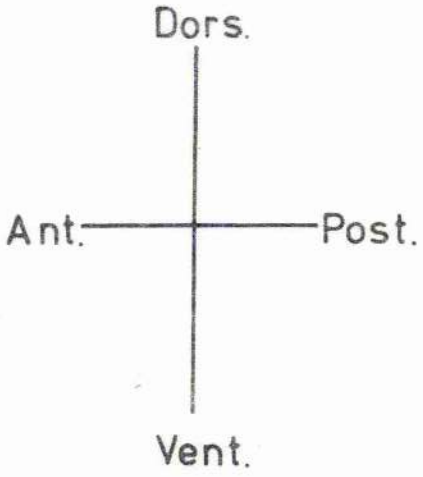
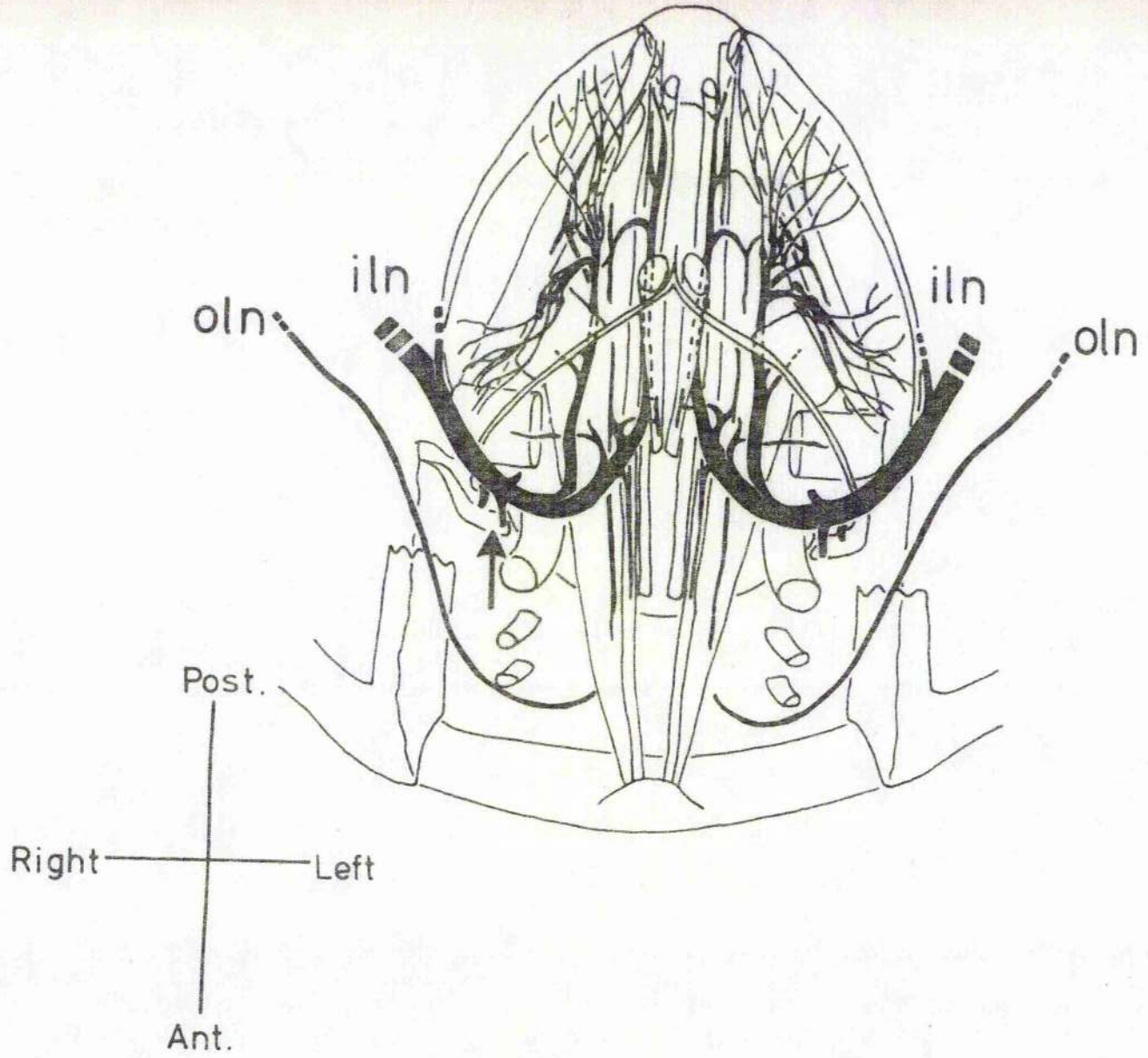

Dors.
Ant.
Post.
Vent.

Figure 10

Labral innervation. Dorsal aspect of the labrum to show the total innervation. O1 and L6 have been removed, and the arrow indicates the region of branching whence they are innervated.

i.l.n. - inner labral nerve

o.l.n. - outer labral nerve


oln
iln
iln
oln
Post.
Right
Left
Ant.

innervating the apron of cuticle which joins the scutiform sclerite to the epistoma.

Sense Organs

As previously mentioned, the lateral root of each i.l.n. innervates three groups of sensory cells (Fig. 11). They are bilaterally symmetrical about the midline and will be designated a,b and c. The cells contained in each group are similar, being large bipolar cells (50-60µm long axis of cell body) with long multiterminal dendrites.

Group a. (Fig. 12 and Fig. 13b) is innervated by a ventral branch of the i.l.n.(L). It comprises 3-6 cells whose dendrites travel ventrally until they reach the floor of the labrum. At this point they branch to pass anteriorly and posteriorly along the floor of the labrum.

Group b. (Fig. 12 and Fig. 13a) has about the same number of cells as group a. The dendrites of its cells pass posteriorly and ramify extensively to innervate a large portion of the labral lobe.

After innervating group b, the i.l.n.(L) turns back on itself to pass laterally around L7 and innervate group c. (Fig. 12 and Fig. 13a). This last group is situated laterally in the labrum at about the same level in the anterior/posterior axis as group a. It is slightly larger than either a. or b. and contains 6-10 neurons which innervate the side of the labrum.

The dendrites of none of the cells described above (from a,b or c) can be seen to interact in any way with the labral cuticle, and no obvious cuticular modifications which might be associated with the dendrites are apparent when the surface of the labrum is viewed with the scanning electron microscope.

In summary, therefore, all those parts of the labrum which are liable to deformation by external forces (viz. the floor, lobe and sides) are innervated by large bipolar sense cells which are contained in three paired groups.

Figure 11

Sensory innervation of the labrum. Medial aspect of the 3 groups of sense cells of the right side of the labrum. Group a innervates the floor, group b the lobe, and group c the side.

i.l.n.(L) - lateral root of the inner labral nerve

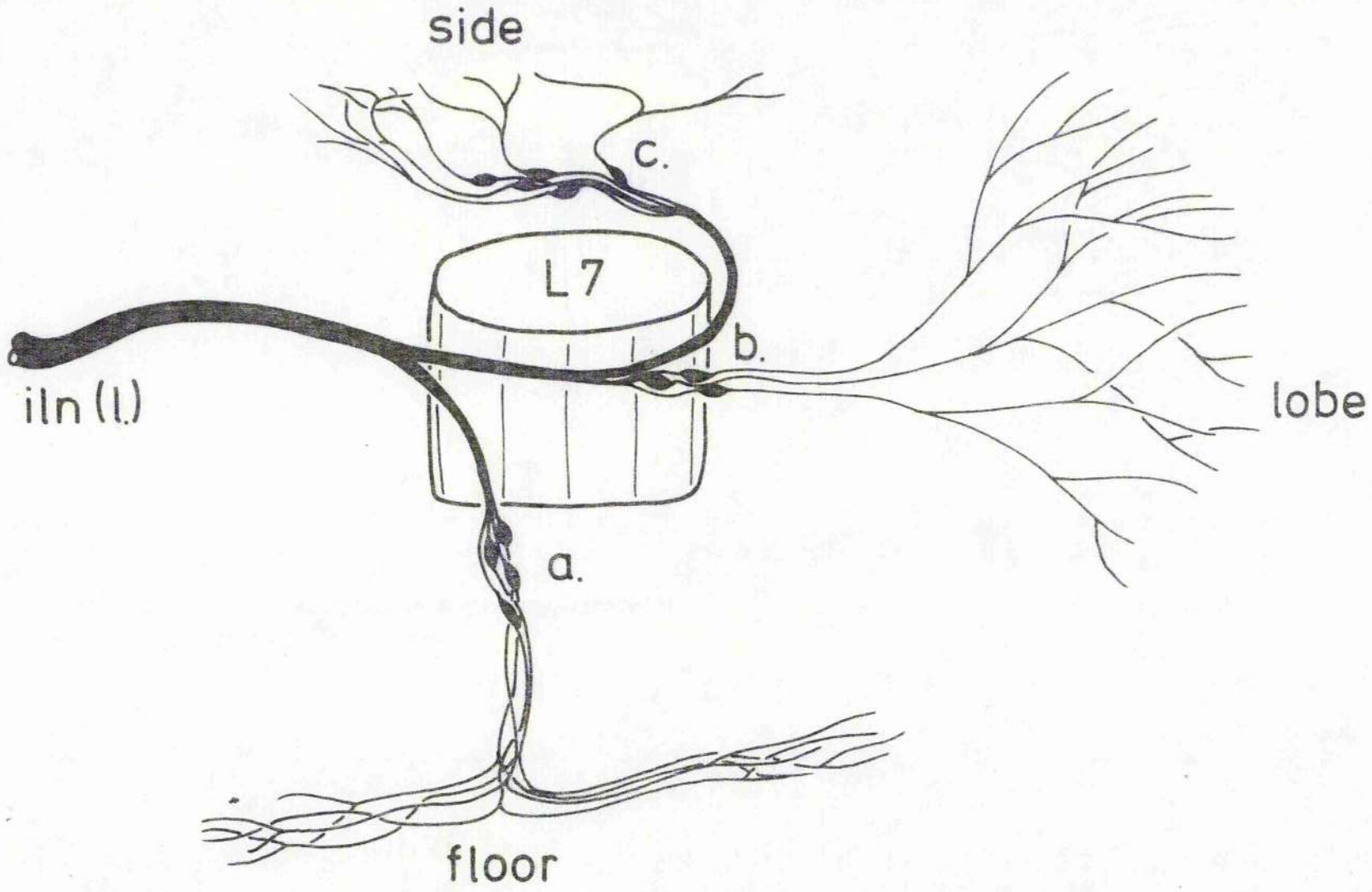

side
c.
L7
b.
iln (l.)
lobe
a.
floor

Figure 12

Sensory innervation of the labrum. Medial aspect of left half of Me. blue stained labrum. Group a innervates the floor, group b the lobe, and group c the side of the labrum. i.l.n.(L) can be seen curving laterally around L7.

Scale mark - 1mm.

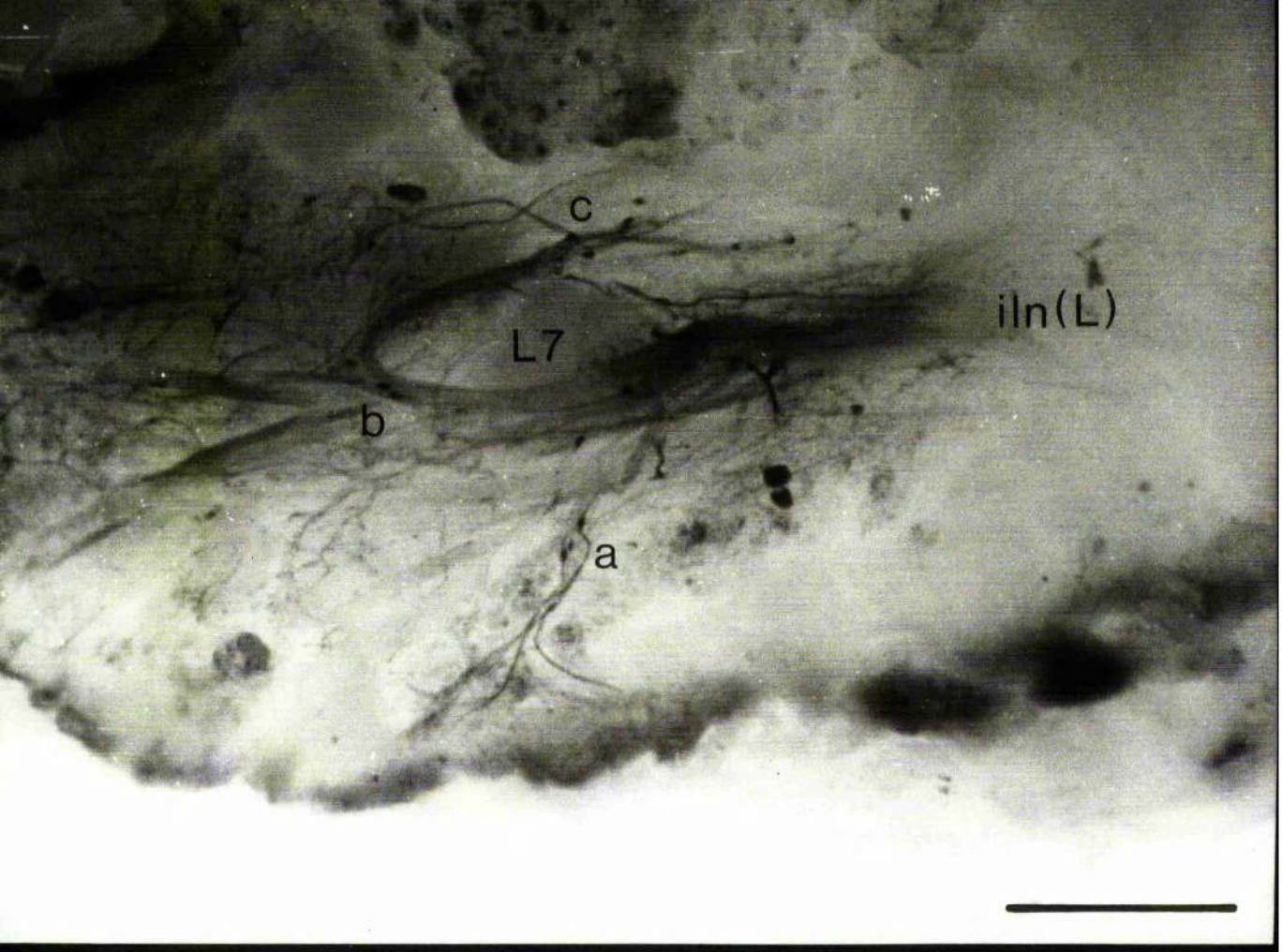
Figure 13

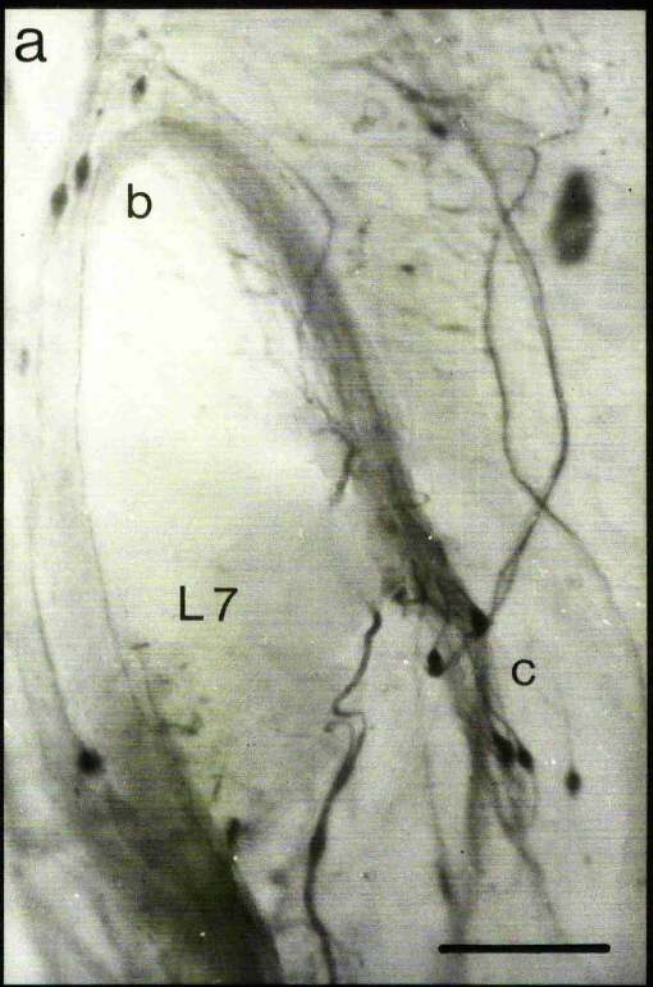
Sensory innervation of the labrum. Me. blue staining.

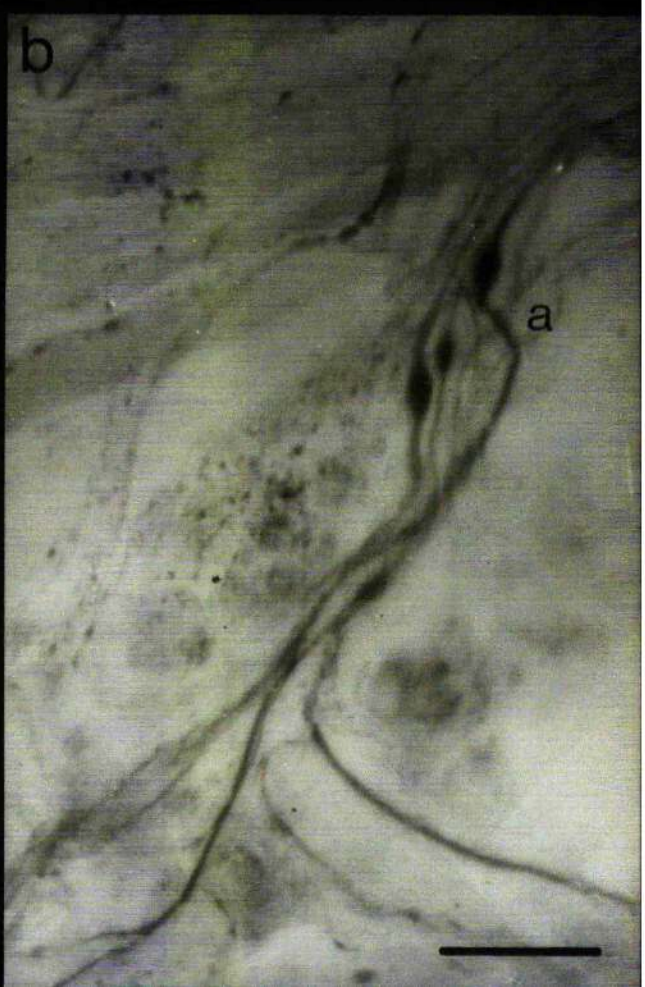
a) Magnification of groups b and c

b) Magnification of group a

Scale marks - 200µm


c
iln(L)
L7
b
a


a
b
L7
c


b
a

B. OESOPHAGUS

Morphology

The oesophagus is a short tube of thin, flexible cuticle connecting the mouth with the cardiac sac. The anterior wall folds inwards to give the lumen a 'U'-shape in transverse section, with the arms of the 'U' pointing anteriorly. This fold, and thus the anterior oesophageal wall, connects ventrally with the furcular sclerite of the labrum. The lateral walls of the oesophagus are attached to grooves on the inner rims of the mandibles. Posteriorly the oesophageal cuticle attaches to the metastomal plate of the ventral skeleton (Snodgrass, 1952), and gives rise to the paragnathe and the 1st maxillae. The dorsal limit of the oesophagus is defined by the oesophageal/cardiac sac valve (Fig. 14). This is a simple valve composed of 4 lobes; one anterior which is continuous with the anterior fold of the oesophagus; two lateral (right and left); and one small posterior lobe which is situated at the antero-ventral limit of the ventral gutter, between the ventral ends of ossicles Xa and XIII (Maynard and Dando, 1974). The posterior lobe would appear to play no major part in occluding the opening between the oesophagus and the cardiac sac. This function is performed by the three remaining lobes (anterior and lateral) which are invested with three pairs of extrinsic dilator muscles (OCEV 1, 2 and 3, see below).

Musculature

Movements and peristalsis of the oesophagus are controlled by four pairs of extrinsic muscles (the dilators), and one complex intrinsic muscle (the contrictor). These are numbered starting with the most antero-ventral, and moving dorsally, laterally and posteriorly. The oesophageal/cardiac sac valve is controlled by 3 pairs of extrinsic dilators and the upper limits of the posterior oesophageal dilator (OEp) and of the oesophageal

Figure 14

Oesophageal/cardiac sac valve

A. Interior aspect from cardiac sac

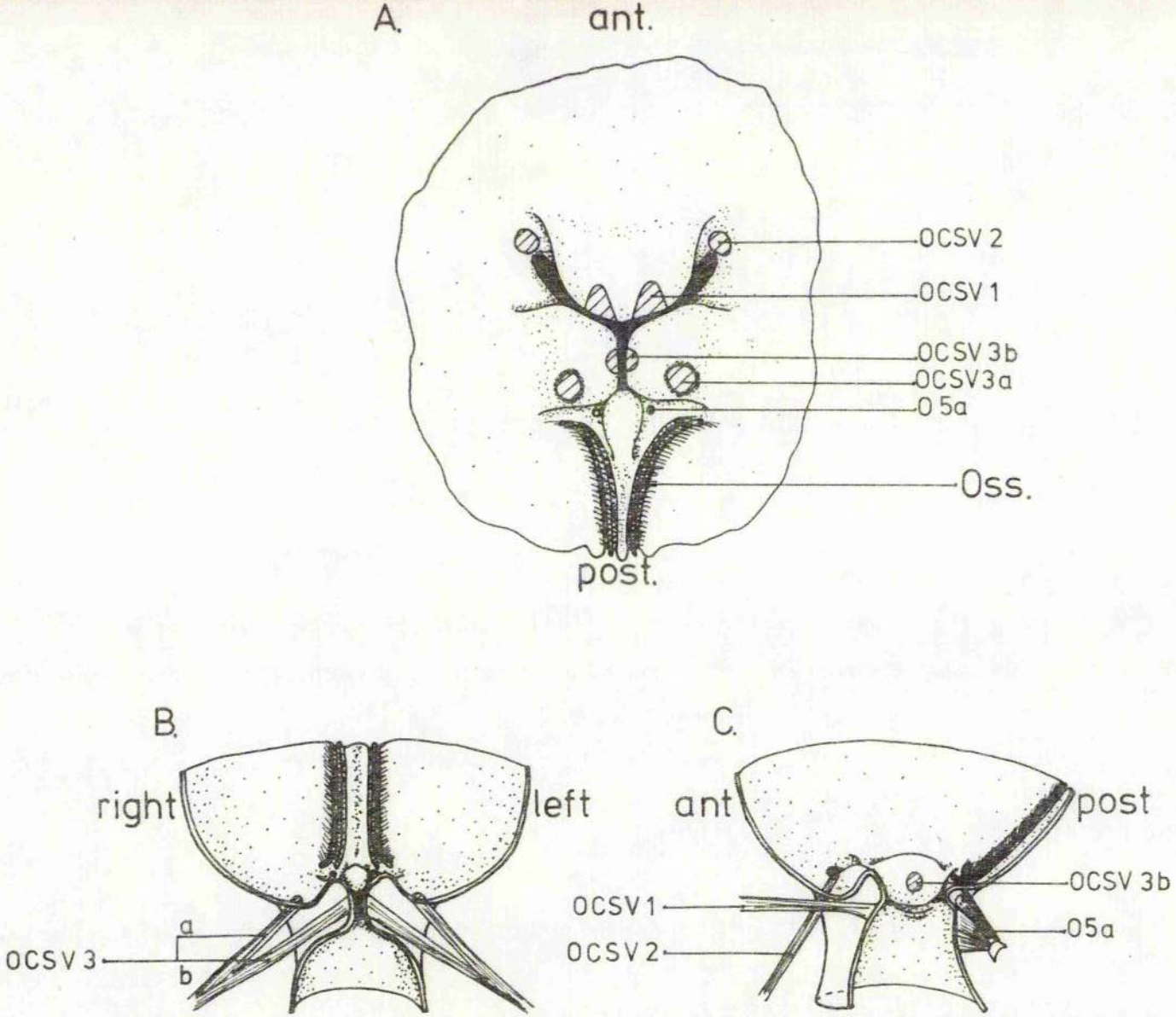
B. Anterior aspect

C. Left-lateral aspect

O5a - oesophageal dilator muscle

OCSV.. - muscles of the oesophageal/cardiac sac valve.
Hatched areas indicate the positions of their insertions.

OSS. - ossicles Na and X111


A.
ant.
OCSV 2
OCSV 1
OCSV 3b
OCSV 3a
O 5a
Oss.
post.
B.
right
left
a
OCSV 3
b
C.
ant
post
OCSV 3b
OCSV 1
O5a
OCSV 2

constrictor (O4). The O.C.C.V. muscles are numbered from medial to lateral. The above muscles are portrayed in Fig. 15 (left lateral aspect), Fig. 16 (anterior aspect) and Fig. 17 (oesophagus split ventrally and flattened). Included in the diagrams are the cardiac sac muscles C4, C5 and C6; the ventral cardiac muscle, CV1; and the cardiopyloric valve muscles, CPV2a and CPV2b. The origins, insertions and routes of these muscles are virtually the same as those described for the homologous muscles in Homarus americanus (Maynard and Dando, 1974) and thus need not be redescribed for H. gammarus. Also shown (Fig. 17) is the paragnathal retractor (Wales, Macmillan and Laverack, 1976 in their Fig. 3), which originates from the medial, anterior cuticle of the ipsilateral mandible.

(a) Oesophageal Musculature

O1 - (see also Figs. 4, 5 and 6 and description of O1a in labral musculature, extrinsic) is the lower anterior oesophageal dilator. It runs from its origin on the median apodeme of the supra-labral ridge, underneath L6 and the oesophageal constrictor (O4), to a large diffuse insertion on the lower anterior wall of the oesophagus. At this insertion the individual muscle fibres intermingle with those of L6. The small bundle (O1a) which enters the anterior oesophageal fold and inserts on the furcular sclerite of the labrum, has already been described.

O2 - is the upper anterior oesophageal dilator. This is a largish muscle originating on the median apodeme of the supra-labral ridge. Its posterior end passes between the fibres of O4 to insert on the anterior oesophageal wall dorsal to the insertion of O1.

O3 - has its origin on the posterior margin of the epistomal plate. It is a large muscle and it runs postero-medially to a broad insertion on the lateral oesophageal wall. The superior oesophageal nerve (s.o.n., see below) runs through the posterior end of O3, effectively separating its

insertion into two heads (upper and lower). This is the lateral oesophageal dilator.

O4 - is the intrinsic muscle of the oesophagus - the constrictor (Fig. 17). Although the majority of this muscle cannot be morphologically divided, there will be functional divisions depending on its innervation pattern. Its various attachments are as follows:-

Extrinsic. (i) 2 paired ventral non-muscular ligaments, anteriorly to the labral falciform sclerite, and posteriorly to the metasternal plate.

(ii) 2 paired ventral muscle branches, anteriorly to the labral furcular sclerite (O1a), and posteriorly to the anterior cuticle of the paragnath.

(iii) A loose posterior connective tissue attachment to the 1st sternal apodeme of the endophragmal skeleton (the cephalic or head, apodeme. Snodgrass, 1952).

Intrinsic. (1) 1 large, diffuse, attachment on the anterior surface of the cardiac sac on either side of the midline and in between the insertions of O5.

(ii) 1 paired dorso-lateral attachment to the cuticular thickening at the insertion of OCSV3a (see below).

(iii) The passage of the fibres of the extrinsic oesophageal and OCSV muscles to their insertion on the cuticle, through the fibres of O4 anchors O4 to the cuticle in three areas. These occur as well defined longitudinal bands anteriorly, laterally and posteriorly (hatched areas in Fig. 17). At these bands the fibres of the extrinsic dilators tend to run longitudinally up and down the oesophagus. It is possible that fibres from O4 also insert on the cuticle in these areas and thus contribute to effective anchorage.

O5 - can be subdivided into upper (O5a) and lower (O5b) components. Both parts have their origins on the cephalic apodeme and the first portion of the major mandibular abductor apodeme (M7 of Wales, Macmillan and Laverack, 1976). O5a has a broad diffuse insertion on the dorsal portion of the posterior oesophageal cuticle. Its limit is defined by small branches at the level of the oesophageal/cardiac sac valve. O5b has insertions directly opposite the cephalic apodeme, it then becomes a broad flat muscle which runs ventrally to insert on the posterior ventral rim of the oesophagus. O5 is the posterior oesophageal dilator.

(b) O.C.S.V. Musculature

OCSV1 - is broad in the dorso-ventral plane and narrow latero-medially. It arises midway along a narrow ligamentous strap which runs between the median apodeme of the supra-labral ridge, and the exoskeleton on the ipsilateral side of the cerebral ganglion. From this origin it passes posteriorly to its insertion in the anterior oesophageal fold, just below the anterior lobe of the oesophageal/cardiac sac valve. Contraction will open the valve and dilate the dorsal limit of the oesophagus.

OCSV2 - originates on the supra-labral ridge lateral to the median apodeme. It is a very narrow muscle which runs postero-dorsally to its insertion on the oesophageal cuticle at the lateral edge of the OCSV's anterior lobe. This muscle will act to open the valve by retracting the anterior lobe.

OCSV3 - is divided into dorsal (OCSV3a) and ventral (OCSV3b) portions. They have a common origin on the posterior margin of the epistoma lateral to the origin of O3 and medial to the common origin of CV1 and C4. OCSV3a inserts on a cuticular thickening in the dorso-lateral corner of the lateral lobe, and its action will be to open the valve by retracting

Figure 15

Oesophageal musculature. Left lateral aspect.

C.. - Cardiac sac muscles

CPV - Cardio-pyloric valve muscles

C.Sac - Cardiac sac

CV.. - ventral cardiac muscle

Endo.Sk. - 1st sternal apodeme of endophragmal skeleton (cephalic apodeme)

Epi. - Epistoma

L.. - Labral muscle

Lig.St. - Ligamentous strap

Mand. - Mandible

O.. - Oesophageal muscles

OCSV.. - Oesophageal/cardiac sac valve muscles

Ossicles - Ossicles Xa + XIII

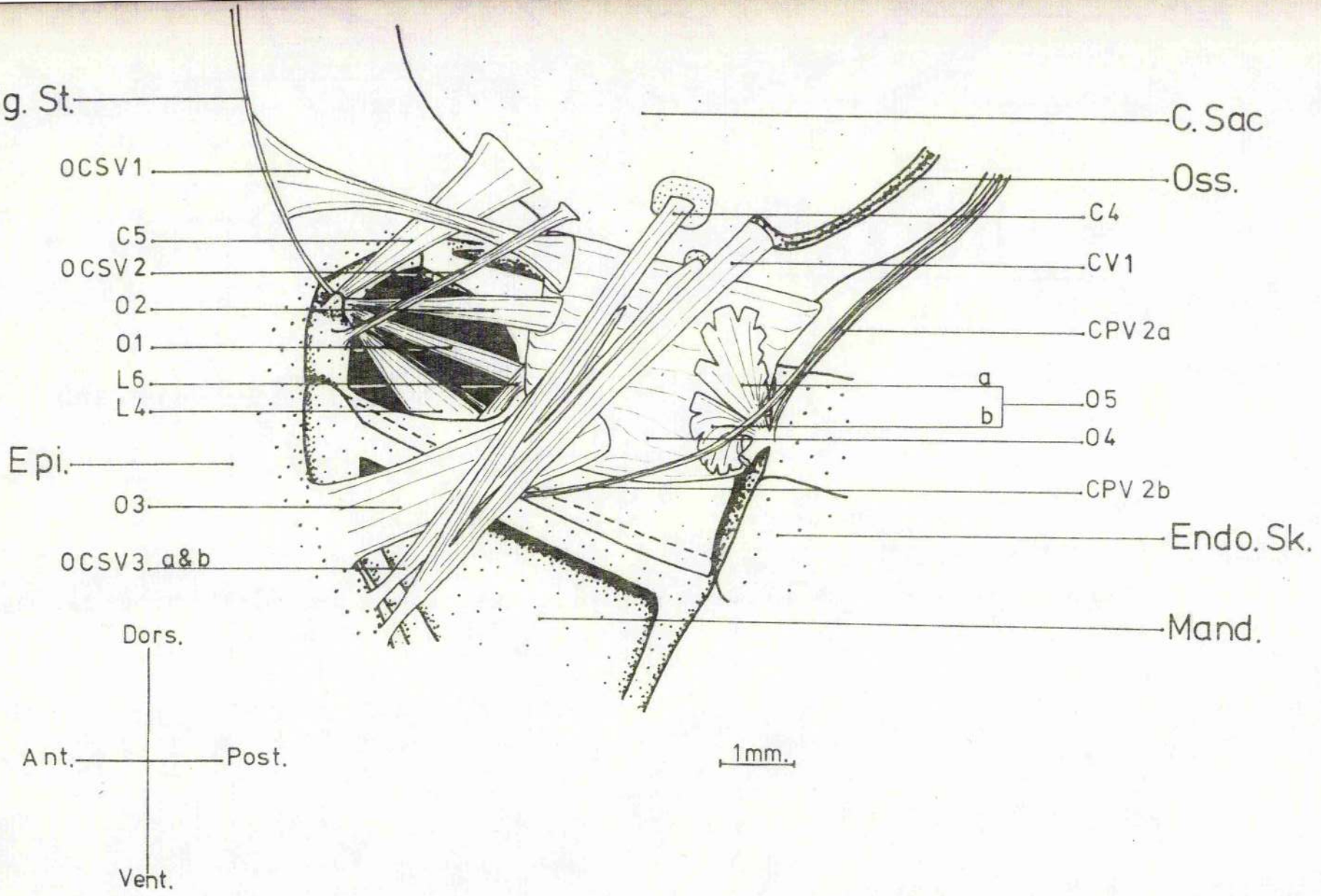

Lig. St.
OCSV1
C5
OCSV2
O2
O1
L6
L4
Epi.
O3
OCSV3 a&b
C. Sac
Oss.
C4
CV1
CPV 2a
a
b
O5
O4
CPV 2b
Endo. Sk.
Mand.
Dors.
Ant.
Post.
Vent.
1mm.

Figure 16

Oesophageal musculature. Anterior aspect

C.. - Cardiac sac muscles

C.Sac. - Cardiac sac

CV.. - Ventral cardiac muscle

L.. - Labral muscle

Lig.t. - Ligamentous strap

O.. - Oesophageal muscles

OCSV.. - Oesophageal/cardiac sac valve muscles

S.L.R. - Supra-labral ridge

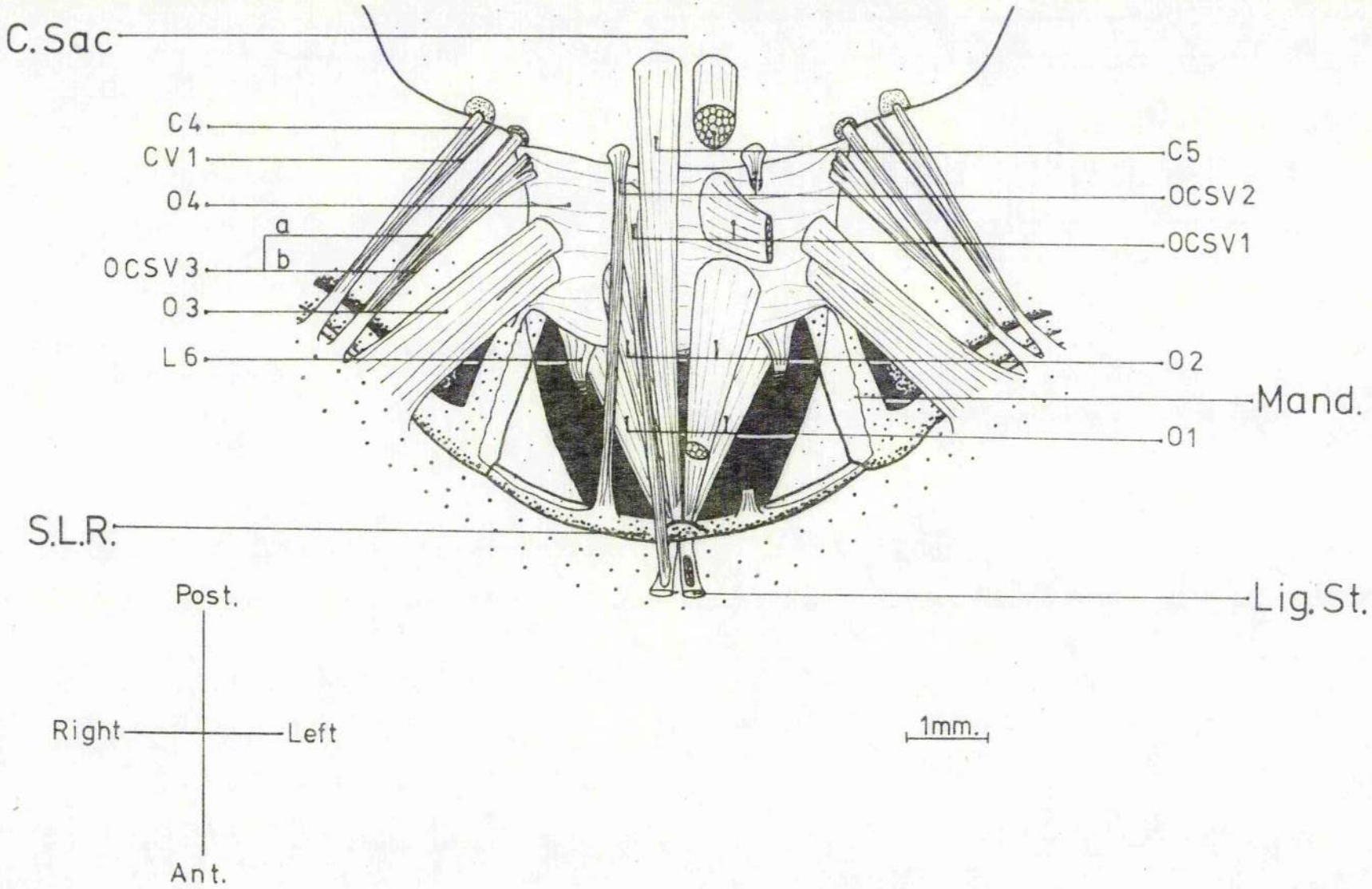

C. Sac
C4
CV1
O4
a
b
OCSV3
O3
L6
C5
OCSV2
OCSV1
O2
Mand.
O1
S.L.R.
Lig. St.
Post.
Right
Left
Ant.
1mm.

Figure 17

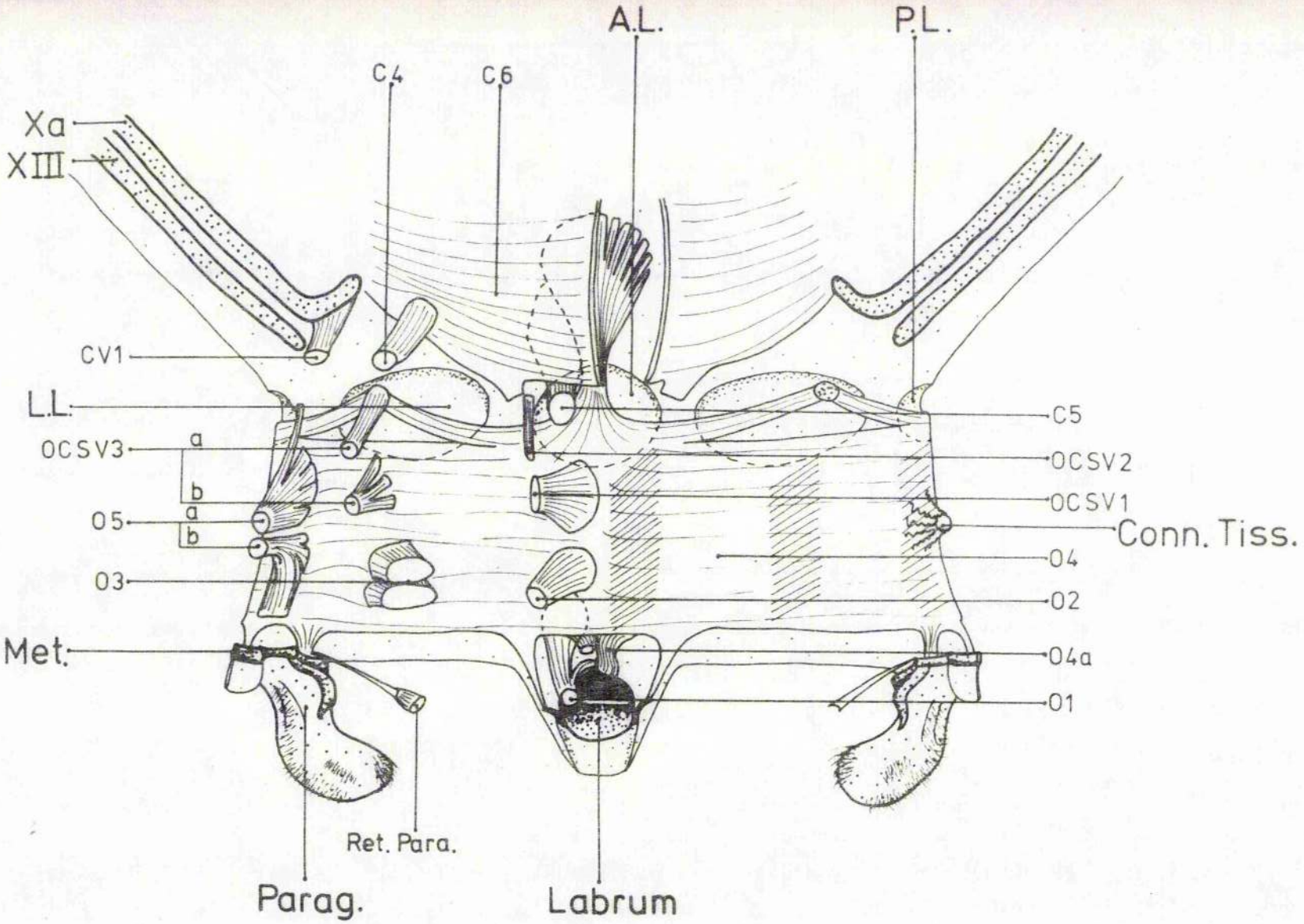
Oesophageal musculature. The oesophagus and the lower portion of the cardiac sac have been split along the posterior midline and flattened. The right side (left side of the diagram) depicts the total musculature, and the other side depicts the intrinsic musculature (O4 and O6)

Note the attachments of O4:

4 paired ventral:- (2 non-muscular ligaments (to falciform sclerite; to metastomal plate)
(2 muscular attachments (to furcular sclerite (O4a); to paragnathal cuticle)

1 median posterior attachment to the cephalic apodeme. (Conn. Tiss.)
1 large median antero-dorsal insertion on the cardiac sac
1 paired dorso-lateral insertion on the cuticular thickening at the insertion of OCSV3a. Hatching indicates where the insertions of the extrinsic oesophageal and OCSV muscles anchor O4 to the oesophageal cuticle, and also provide bands of intrinsic longitudinal muscle fibres.

A.L. - anterior lobe of OCSV
C.. - cardiac sac muscles
Conn.Tiss. connective tissue attachment to cephalic apodeme
CV1 - ventral cardiac muscle
L.L. - lateral lobe of OCSV
Met. - metastomal plate
O.. - oesophageal muscles
OCSV.. - muscles of oesophageal/cardiac sac valve
Parag. - paragnath
Ret.Para.- retractor paragnatha
Xa + X111 ossicles of Cardiac sac


A.L.
P.L.
C4
C6
Xa
XIII
CV1
L.L.
OCSV3
a
b
05
a
b
03
Met.
C5
OCSV2
OCSV1
Conn. Tiss.
04
02
04a
01
Ret. Para.
Parag.
Labrum

this lobe. OOV3b has a diffuse insertion on the ventral border of the lateral lobe and on the oesophagus ventral to this. It can be described as an upper lateral oesophageal dilator.

04 - Although closure of the valve will be passively induced by the relaxation of OOV 1, 2 and 3 and by the weight of food material in the cardiac sac, 04 will play a significant part. It can be seen that the dorsal limit of 04 consists of a band of muscle running around the oesophagus at the level of the valve and inserting anteriorly, laterally and posteriorly. Contraction will constrict the valve and thus effect closure.

Innervation

The oesophageal nervous system is shown diagrammatically in Figures 18, 19 and 20, and very schematically in Fig. 21. The nomenclature used follows Maynard and Dando (1974) with one exception. They describe in Callinectes sapidus, Homarus americanus and Panulirus argus the postero-lateral nerve (p.l.n.) arising as a fusion of the dorsal posterior oesophageal nerve (d.-p.o.n.) and the ventral posterior oesophageal nerve (v.-p.o.n.). In Homarus gammarus there appears to be little, if any, fusion between the corresponding nerves, and so the p.l.n. is considered as arising directly from the superior oesophageal nerve (s.o.n.), and a d.-p.o.n. section is not described. The courses of the major nerves in this area have been well described in a number of animals (Allen, 1894; Heim, 1915; Nocquard, 1883; Paterson, 1968; Pearson, 1908), but they are described here to provide a framework upon which to base a more detailed description. Small nerve fibres innervating specific muscles have not been named as it may be better to wait until more is known about the motoneurons travelling in them.

(a) General Layout

The motor innervation of the labral/oesophageal complex originates from the paired commissural ganglia (Co.Gs.) and the oesophageal ganglion (O.G.). The commissural ganglia are situated ventrally on the circum-oesophageal connectives (C.Cs.) which run between the cerebral ganglia (Ce.Gs.) and the suboesophageal ganglion (S.G.) on either side of the oesophagus. Posterior to the oesophagus and anterior to the S.G. the connectives are joined by the post-oesophageal commissure (p.o.c.c.). The commissural ganglia give rise to three major nerve trunks:- the inferior oesophageal nerves (i.o.ns.); the superior oesophageal nerves (s.o.ns.); and the ventral-posterior oesophageal nerves (v.-p.o.ns.). The i.o.ns. travel medially from the Co.Gs. on the anterior surface of the oesophagus, to meet at the oesophageal ganglion which lies in the anterior midline. The s.o.ns. travel in the same way, dorsal to the i.o.ns., and meet in the anterior midline. From this junction there arises the short thick oesophageal nerve (o.n.) which connects ventrally with the i.o.n./O.G. junction, and the stomatogastric nerve (st.n.) which travels dorsally to connect the oesophageal nervous system with the stomatogastric nervous system. The v.-p.o.ns. run dorsally from the Co.Gs. and come out of the dorsal surface of the circum-oesophageal connectives. From there they run dorsally and posteriorly on the surface of the oesophagus and terminate in sensory endings at the level of the G.C.-.V.

Issuing from the oesophageal ganglion are four major trunks. The i.o.ns. and o.n. are described above. From its antero-dorsal surface the O.G. produces a fine nerve, the inferior ventricular nerve (i.v.n.) which passes anteriorly, between the ligamentous straps, to the cerebral ganglia.

(b) Commissural Ganglia

These are reasonably large ganglia, and apart from the i.o.n., s.o.n. and v.-p.o.n., each Co.G. produces two smaller nerves. One

innervates the lower bundle of O3, the lateral oesophageal dilator, and the other runs ventrally to innervate the lower lateral and posterior region of O4, the oesophageal constrictor.

(c) Oesophageal Ganglion

This is a loosely packed collection of about 15 neurons present at the junction of the i.o.n.r., i.v.n. and o.n. The majority of the cell bodies are situated in a slight swelling at this junction, but it is not uncommon to find some a short distance into the nerve roots. Also common is the presence of two neurons in the i.v.n. approximately midway between the O.G. and the cerebral ganglia. These neurons are monopolar with their axons running towards the O.G. It is doubtful whether their presence is associated with a reduction in the number of neurons in the O.G. Small nerve branches from the O.G. area innervate O2 (right and left) and the lower anterior region of O4.

(d) Inferior Oesophageal Nerve

The majority of fibres in the i.o.n. appear to deal with the labrum and lower oesophagus. It arises from the most ventral point of Co.G. The first branch from it is usually the outer labral nerve (o.l.n.), although this can pass directly into the commissural ganglion without connecting with the i.o.n. The o.l.n. runs antero-medially towards the labrum and innervates M41 before entering the lumen of the labrum. It has already been described (see the section on the labral innervation).

At the level of O1, the i.o.n. bifurcates, sending a large ventrally directed branch, the inner labral nerve (i.l.n.), into the labrum. This innervates O1 and M6 (the lower anterior oesophageal dilator and the labral levator) before passing into the lumen of the labrum to innervate the remaining labral musculature (see above). The i.l.n. also contains a few fibres from

the C.G. area. Variation between animals is such that at one extreme the fibres from the C.G. into the labrum can be considered as a separate nerve, and at the other extreme they travel in i.o.n. and leave it in i.l.n. with the fibres from the Co.G. Just before reaching the C.G., i.o.n. gives off a small branch which innervates O2, the upper oesophageal dilator.

(e) Superior Oesophageal Nerve

The s.o.n. is a substantial nerve which exits anteriorly from the Co.G. and passes through the middle of O3, separating it into two bundles, before continuing to the s.o.n./o.n./st.n. junction. As it goes through O3, the s.o.n. gives off a small branch dorsally to innervate the upper bundle of this muscle. Just after this a larger nerve branches ventrally from the s.o.n. and runs ventrally and medially to innervate the lower anterior region of O4. Approximately midway along the s.o.n. is a region where four nerves originate. These are: the postero-lateral nerve (p.l.n.); a ventral branch which innervates the anterior mid-region of O4; a small anterior branch which innervates CCCV2 as it runs in front of the s.o.n. to its insertion on the lateral corners of the CCCV's anterior lobe; a sensory nerve which serves the anterior oesophageal sensor (A.O.S.) (see below). Individual variation may alter the relative positions of the origins, from the s.o.n., of these nerves.

The p.l.n., as its name suggests, runs posteriorly and laterally from the s.o.n. It passes through muscle CV1 and continues to become part of the stomatogastric nervous system. On the way it gives off a medial branch which innervates the upper anterior region of O4, and two lateral branches which innervate CCCV3a and b.

Just before reaching the midline, the s.o.n. gives off a small nerve to innervate CCCV1. This muscle is also innervated from the stomatogastric nerve (st.n.) as is C5.

Figure 18

Oesophageal innervation. Left lateral aspect. The extrinsic muscles are indicated by the positions of their insertions. The arrow indicates the branch from the s.o.n. which innervates OCCV2 as it passes in front of this nerve.

A.O.S.	-	anterior oesophageal sensor
C..	-	cardiac muscle
C.C.	-	circum oesophageal connective
Co.G.	-	commissural ganglion
CV1	-	ventral cardiac muscle
iln	-	inner labral nerve
ion	-	inferior oesophageal nerve
ivn	-	inferior ventricular nerve
MPR1	-	mouth part receptor 1
O..	-	oesophageal muscle
OCCV..	-	oesophageal/cardiac sac valve muscle
O.G.	-	oesophageal ganglion
oln	-	outer labral nerve
on	-	oesophageal nerve
pln	-	posterc-lateral nerve
poc	-	post oesophageal commissure
P.O.S.	-	posterior oesophageal sensor
son	-	superior oesophageal nerve
stn	-	stomatogastric nerve
v-pon	-	ventral-posterior oesophageal nerve

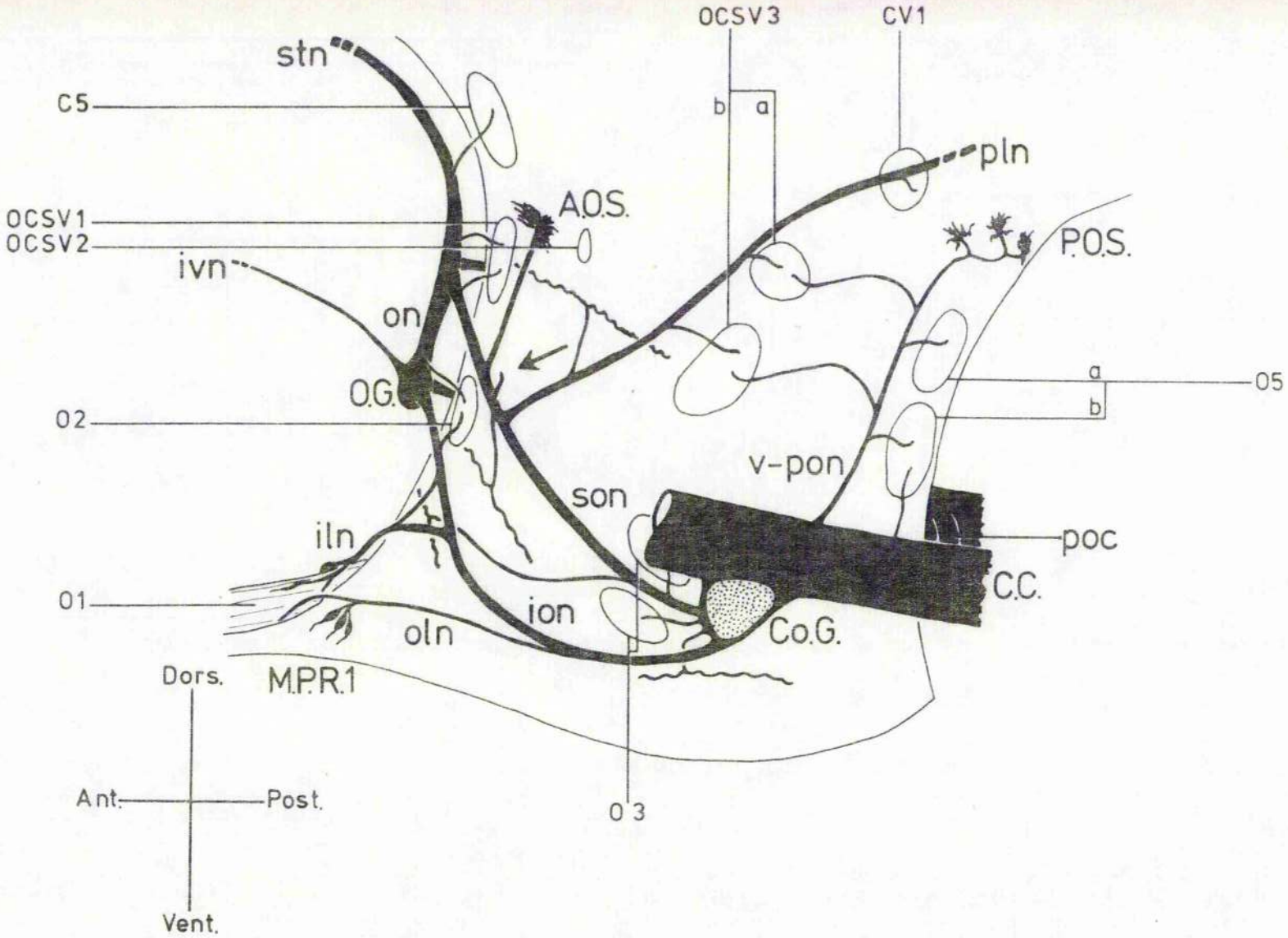

OCSV3
CV1
stn
C5
b
a
pln
A.O.S.
OCSV1
OCSV2
P.O.S.
ivn
on
a
05
O.G.
b
02
v-pon
son
poc
iln
C.C.
01
ion
Co.G.
oln
Dors.
M.P.R.1
Ant.
Post.
03
Vent.

Figure 12

Oesophageal innervation. Antero-dorsal aspect. The extrinsic muscles are indicated by the positions of their insertions. The arrow indicates the branch from the s.o.n. which innervates OOV2 as it passes in front of this nerve.

Abbreviations as before

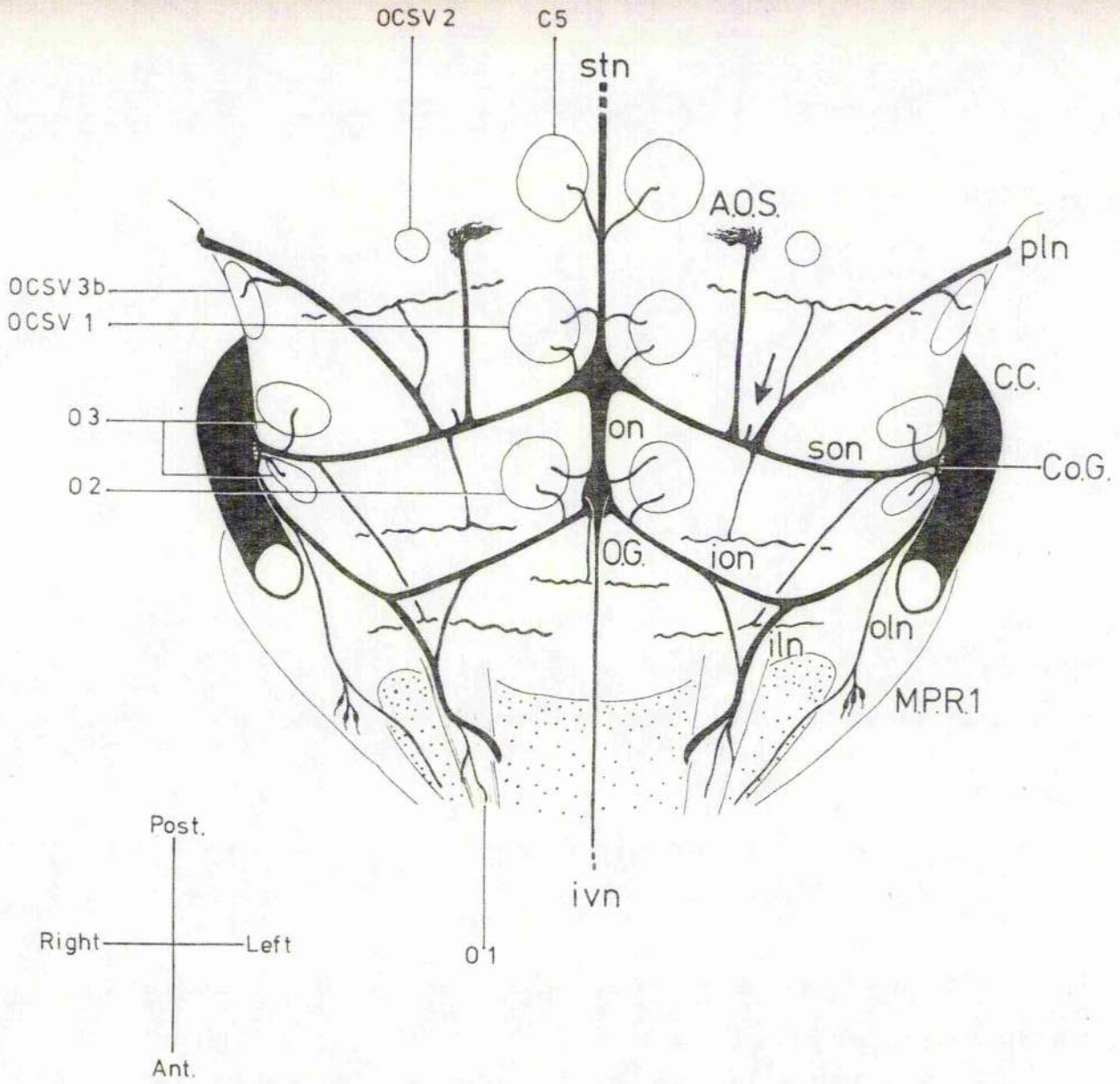

OCSV 2
C5
stn
A.O.S.
pln
OCSV 3b
OCSV 1
C.C.
03
on
son
Co.G.
02
O.G.
ion
oln
iln
M.P.R.1
ivn
01
Post.
Right
Left
Ant.

Figure 20

Oesophageal innervation. The oesophagus and the lower portion of the cardiac sac have been split in the posterior midline and flattened. The right side (left side of the diagram) depicts the innervation of the extrinsic musculature and the other side depicts the innervation of the intrinsic musculature (04).

Abbreviations as before

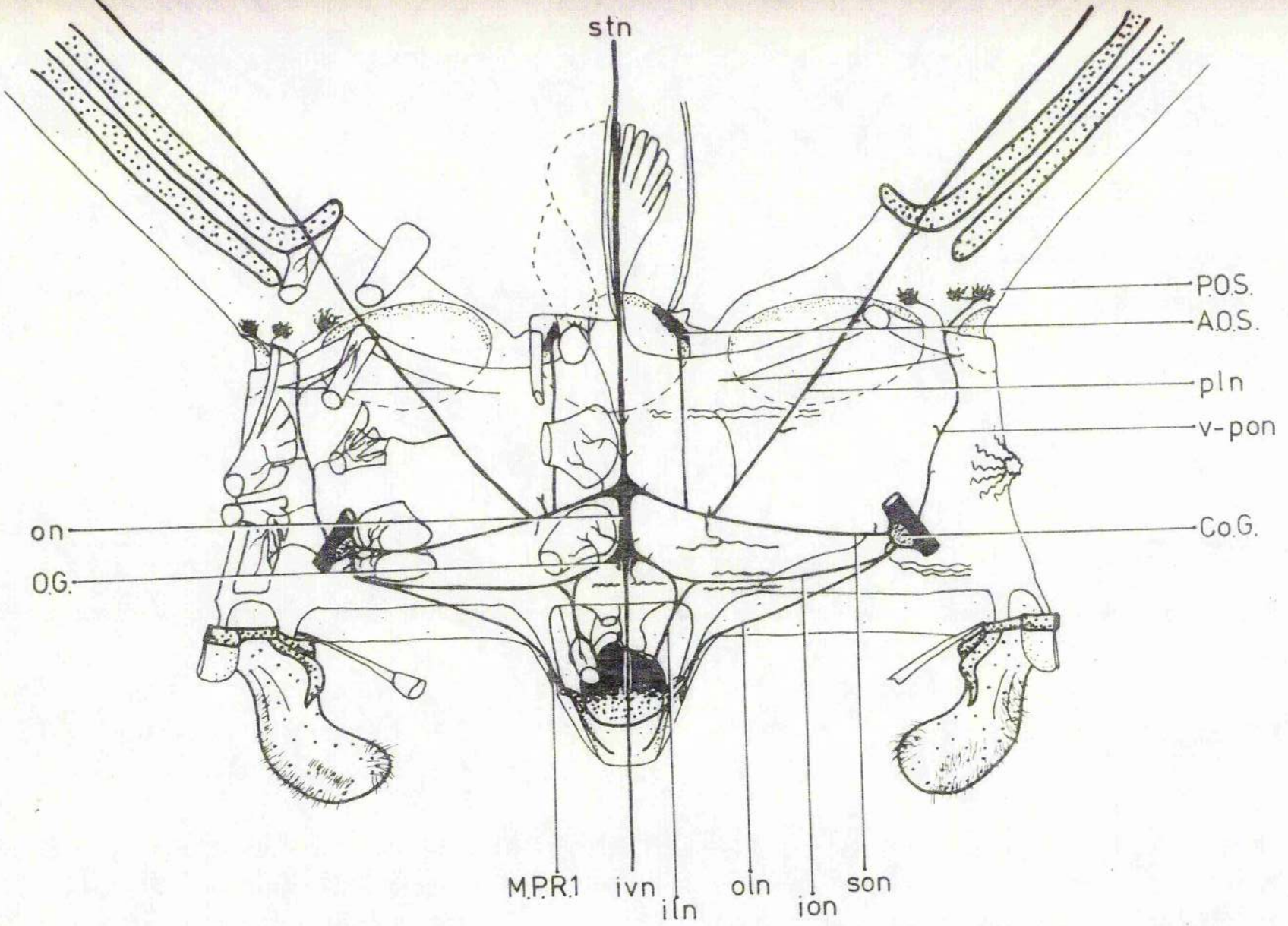

stn
P.O.S.
A.O.S.
pln
v-pon
Co.G.
on
O.G.
M.P.R.1
ivn
iln
oln
ion
son

Figure 21

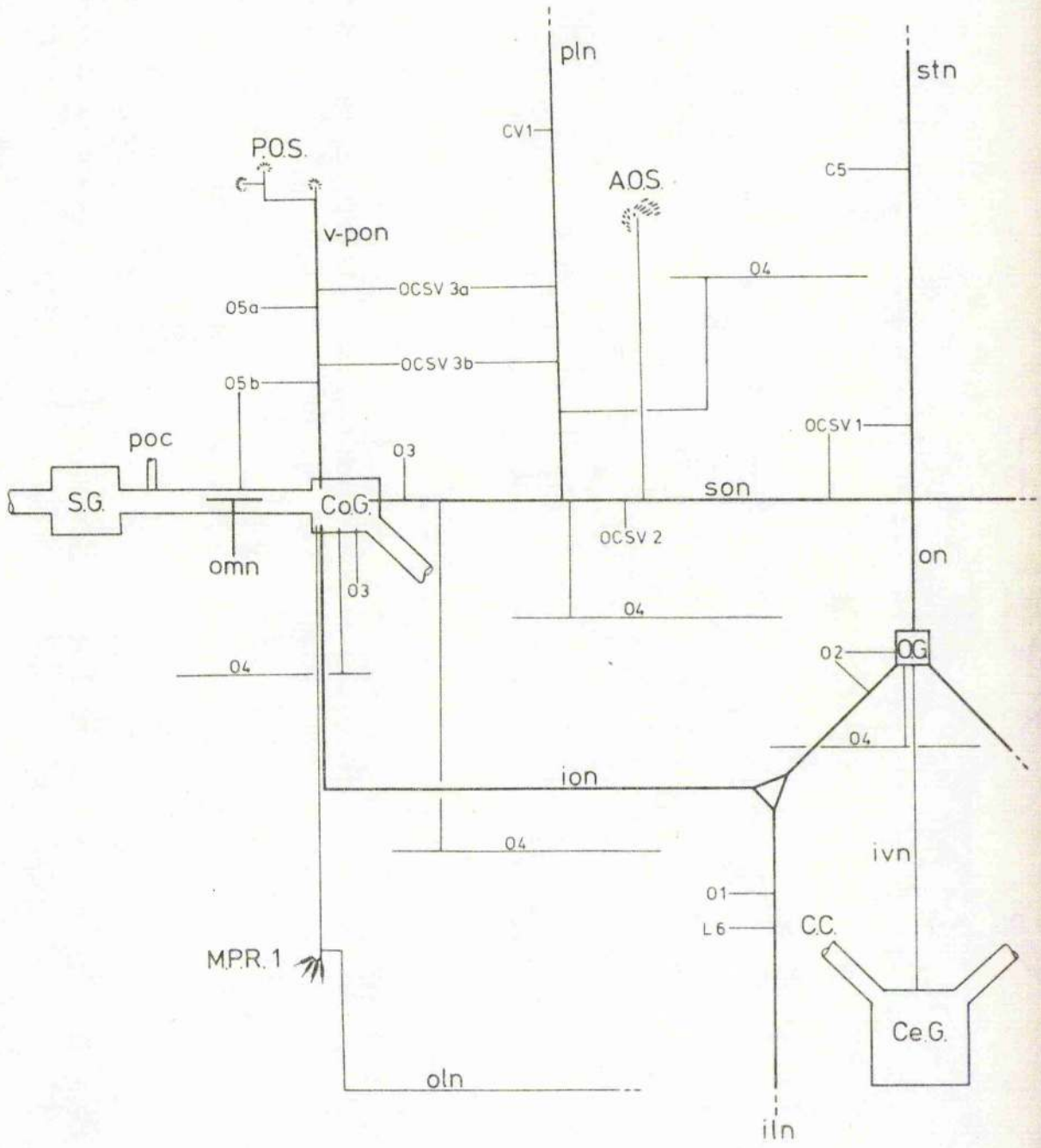
Schematic representation of the innervation of the right side of the oesophagus.

Abbreviations as before plus:-

Ce.G. - Cerebral ganglia

oun - outer mandibular nerve

S.G. - Suboesophageal ganglion


pln
stn
CV1
C5
P.O.S.
A.O.S.
v-pon
04
OCSV 3a
05a
OCSV 3b
05b
OCSV 1
poc
03
S.G.
CoG.
son
OCSV 2
on
omn
03
04
04
OG
02
04
ion
04
ivn
01
L6
C.C.
M.P.R. 1
Ce.G.
oln
iln

(f) [illegible]ntral - Posterior Oesophageal Nerve

Before terminating in the three groups of sensory endings of the posterior oesophageal sensor (P.O.S.), the v.-p.c.n. sends off small branches to innervate O5 V3a and b (the upper lateral oesophageal dilators), and O5a and b (the posterior oesophageal dilators). O5b is also innervated by a small nerve from the circum-oesophageal connective, posterior to the Oe.G.

C. ORGANS OF THE OESOPHAGEAL/CARDIAC SAC VALVE

The Anterior Oesophageal Sensors

The anterior oesophageal sensors (A.O.S.) are crescent-shaped organs situated on the oesophagus on either side of the anterior midline and at the level of the G.C.N. They are symmetrical with each other and occur at the lateral limits of the anterior lobe (A.L.) of the O.C.S.V. The position of the sensors in relation to the nervous system of the whole oesophagus has been shown in Figs. 18, 19, 20 and 21 (see section on oesophageal innervation).

(a) Methylene blue

Fig. 22 and its inset are diagrams of the A.O.S. from anterior and lateral aspects based on information obtained from Me. blue permanent preparations (Fig. 23a,b & c). On preliminary examination, each sense organ can readily be divided into two populations of receptor cells (a + b in Figs. 22 and 23a). Group a (Fig. 23b) is composed of 250-300 small (15-20µm - long axis of cell body) bipolar neurons. They have short dendrites and are situated in a narrow band at the lateral edge of A.L.

The second population, group b, consists of 50-60 larger structures which innervate a wide curving band along the dorso-lateral border of A.L. Closer investigation reveals each one of these to be a bundle of 3-5 small (15-20µm long axis of cell body) bipolar neurons. Thus the total number

of neurons in group b lies between 150 and 300. The neurons in each bundle are closely associated and they tend to stain as a single structure. The underlying composition was revealed in preparations which had either stained poorly or destained during fixation and mounting. When the preparations are viewed with Nomarski-interference-contrast illumination the dendrites of each bundle can be seen to be associated with discrete structures on the internal surface of the oesophagus (Fig. 24a and b). These were examined further with the scanning electron microscope (see below). The axons of both group a and group b neurons travel ventrally in a large bundle (the a.o.c.n.) and join the c.o.n.

The area around the A.V. is heavily invected with connective tissue (C.T. in Fig. 22 inset) in which the A.C.V. is embedded. This connective tissue forms a bridge between the cardiac sac and the oesophagus and effectively occludes the opening to the lumen of A.V. It also ramifies around the various muscle insertions, particularly those of CCVV2 and C4.

There is a further group of neurons whose axons also travel in a.c.o.n. to c.o.n. but which is not classified as being part of the anterior oesophageal sensor. This is a small number (2-3) of large (60-80µm long axis of cell body) bipolar neurons present a short distance dorsal to the A.C.V. (Fig. 22 and Fig. 23c). Their cell bodies are not always in close proximity with one another, and their dendrites are long (several mm), and unbranched for as far as they could be traced. The dendrites travel over the surface of the cardiac sac and oesophagus in the region of the C.C.C.V.

(b) Histology

Fig. 25 shows photographs of 1µ sections through the anterior wall of the oesophagus in the region of the A.C.V. Although it is not clear whether the outermost epicuticular layer (0.5µm thick) is penetrated, it is evident that the chitin layer (0.50µm thick) is penetrated in two distinct

ways. Firstly by small pores which are between 1 and 3μm in diameter. These contain stained filaments which run from the epithelium and whose distal ends appear to be associated with small nodules on the epicuticular surface. Secondly by larger pores (5-8μm diameter) which occur in regions where the chitin layer has thinned considerably (down to c.20μm). These large pores are associated with depressions (c.5μm deep and c.20μm in diameter) of the epicuticle and a concomitant thinning of the epicuticular layer. Several filaments of the type seen in the small pores can be seen entering each large pore, and the underlying epithelium seems to be structured in a globular fashion.

The distribution and number of both types of pore were studied in serial sections and this is shown in Fig. 26. There is a large number of small pores and they are confined to a sharply delineated band about 800-1000μm long and c.100μm wide. The larger pores are fewer in number and distributed with a concentration towards the dorsal end of the organ. The area to which they are confined is not as narrowly defined as that of the small pores.

(c) Scanning Electron Microscopy

The internal surface of the oesophagus in the region of the A.L. is heavily invested with a large number of cuticular hairs ranging in length from 200 to 500μm and with a basal diameter of 8-10μm. However at each dorso-lateral corner of the A.L. is a crescent-shaped area which is devoid of these long hairs. These areas correspond exactly with the positions of the A.C.S. as demonstrated with Ne. blue.

Fig. 27 (a and b) shows the left and right (respectively) sides of the A.L. from a ventral aspect. Immediately obvious on the lateral walls of the A.L. are numerous small, rounded hillocks whose basal diameters are approximately 50μm and which appear to have small depressions in their centres.

Closer examination of the whole area, moving laterally over the A.L. from the medial edge of the bare patch, reveals the following structures.

1. 50-70 of the rounded hillocks mentioned above. These commonly have depressions, 10-15μm across, on their raised surfaces. Associated with each depression is a variable small number (1-4) of small nodules (2-4μm diameter). Occasionally small depressions can be seen in the centre of each nodule (arrowed in Fig. 28b). Between the hillocks is a sparse covering of bristles which are 7-10μm long and 1-2μm in diameter. The hillocks themselves are devoid of hairs (see Fig. 28a and b).

2. Lateral to the hillocks is a long narrow area which is also clear of hairs. The epicuticle in this region is not obviously structured in any way, save for a profusion of small nodules similar in size and shape to those described above. In this case, however, they are present singly and do not form recognisable groups. (see Fig. 29a and b).

3. Running alongside this is a narrow band (40-50μm wide) with a dense covering of bristles. Amongst these can occasionally be seen a single line of pits, or pores in the cuticle. These are typically 4-6μm across.

The bristle-band described above marks the lateral angle of the A.L., and the remaining area of the bald patch does not appear to be structured in any way. The information described above is summarised in Fig. 30.

Figure 22

Right anterior oesophageal sensor, anterior aspect. Note two groups of receptor material (a & b) comprising the organ, also a small group (two shown) of large bipolar cells whose axons also run in the a.o.s.n.

A.L. - Anterior lobe of oesophageal/cardiac sac valve
A.O.S. - Anterior oesophageal sensor
aosn - nerve innervating the A.O.S.
O4 - oesophageal constrictor
OCSV2 - a dilator of the oesophageal cardiac sac valve

Inset - Lateral view of the A.O.S. displayed in longitudinal section to show the a.o.s.n. entering A.L. to innervate its walls. Also the profusion of connective tissue bridging the opening of A.L.

C.T. - connective tissue
Oes. - oesophagus

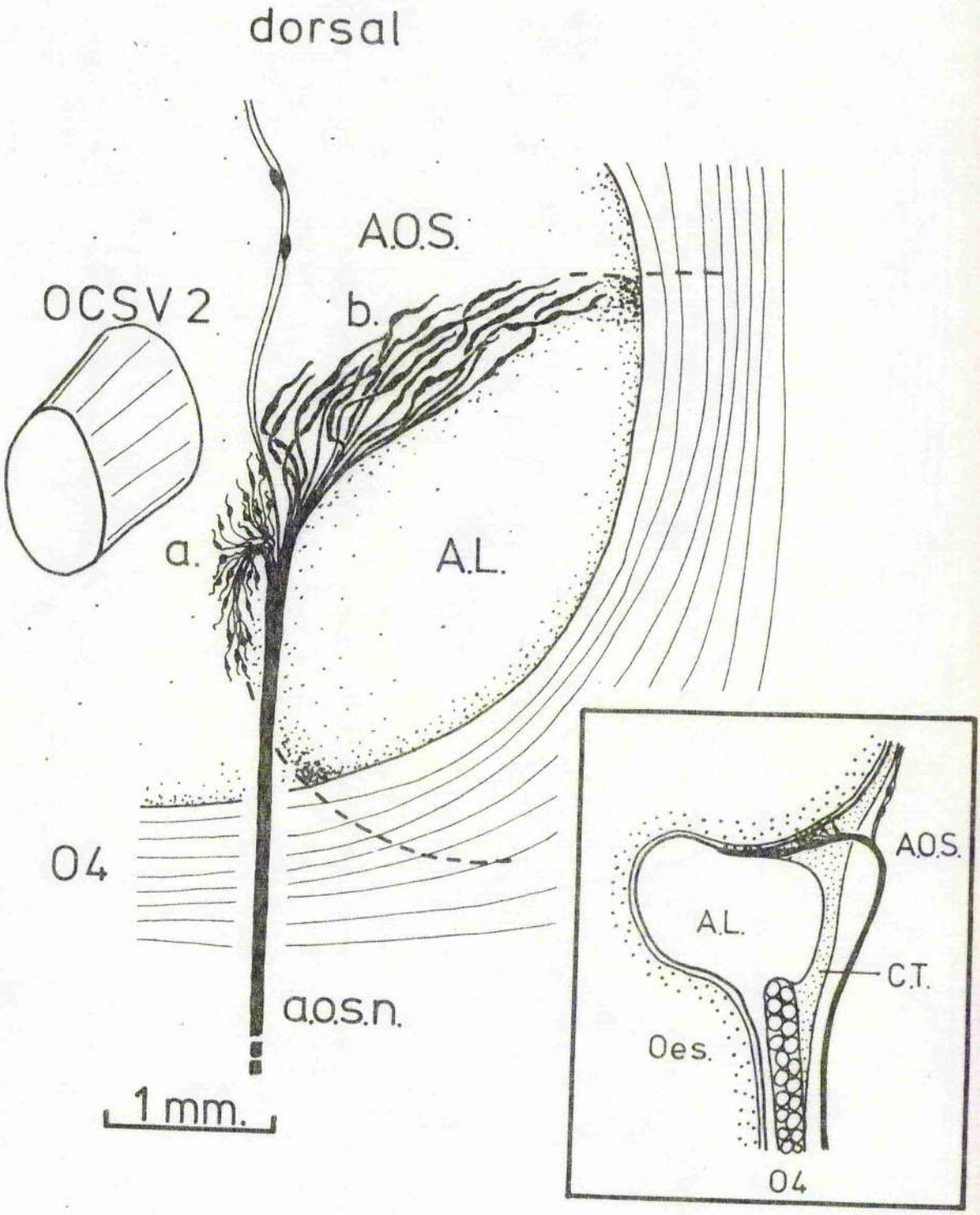

dorsal
A.O.S.
OCSV 2
b.
a.
A.L.
O4
a.o.s.n.
1 mm.
A.O.S.
A.L.
C.T.
Oes.
O4

Figure 23

Methylene blue staining of the right anterior oesophageal sensor.

a. Whole organ. Note two groups of receptor material. Group a - numerous small bipolar cells. Group b - several larger structures which are bundles of 3-5 small cells. Scale mark - 500µm. a.o.s.n. - nerve innervating A.O.S.

b. Enlargement of group a. Scale mark - 300µm.

c. Two bipolar cells whose dendrites travel over the surface of the oesophagus and cardiac sac, and whose axons run in a.o.s.n. Scale mark - 300µm.

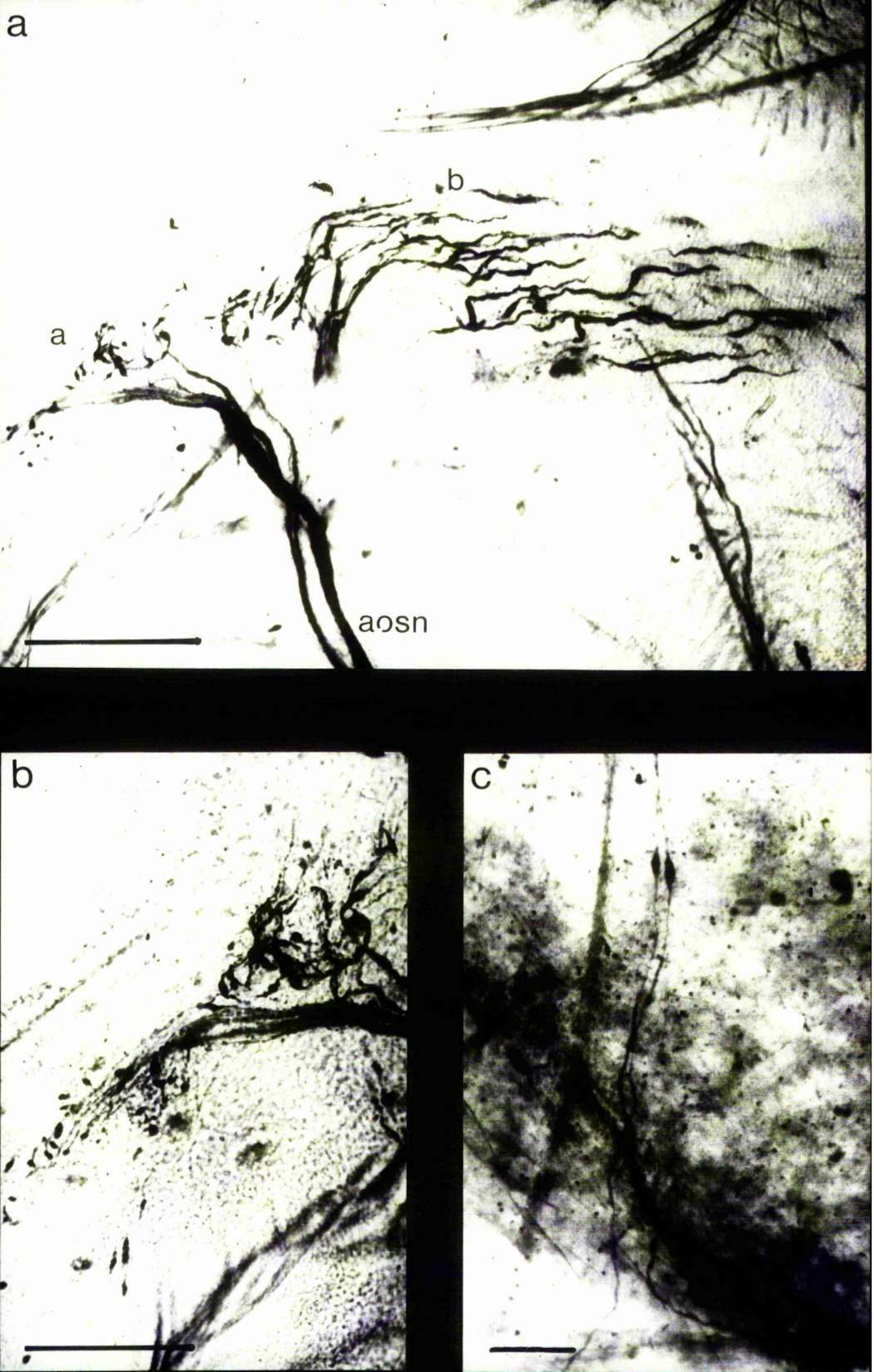

a
b
a
aosn
b
c

Figure 24

Methylene blue stained preparation of the A.O.S. viewed with Nomarski - interference - contrast illumination.

a. - Focussed on a dendrite from one of the bundles of group b.

b. - Same area focussed on the surface of the oesophagus to show the discrete structure (arrowed) associated with the dendrite.

Scale mark - 100µm

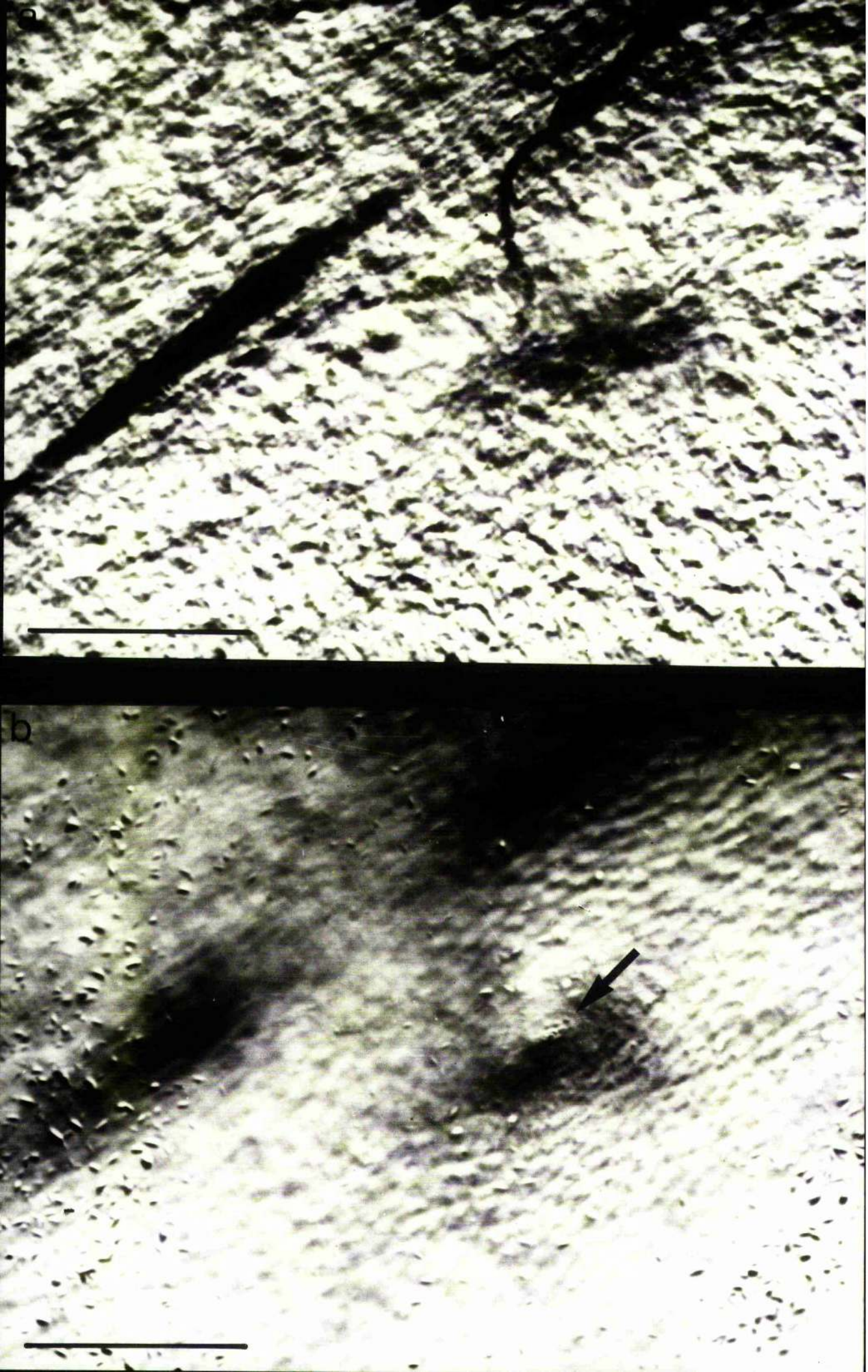

a
b

Figure 25

10µ wax sections through the oesophagus in the region of the A.O.J. stained with Mallory's triple stain. Scale mark - 50µm.

Upper pair - Large pores (indicated by dots) through the chitin layer. These are associated with a thinning of the chitin and epicuticular layers and a depression of the epicuticle. Note also the globular structuring of the epithelium.

Lower pair - Small pores (indicated by dots) through the chitin layer. Stained filaments travelling through the pores from the epithelium are associated with small nodules on the epicuticular surface. No recognisable structuring of the epithelium.

The small pores are tightly grouped in contrast to the larger ones which are present in a wide band.

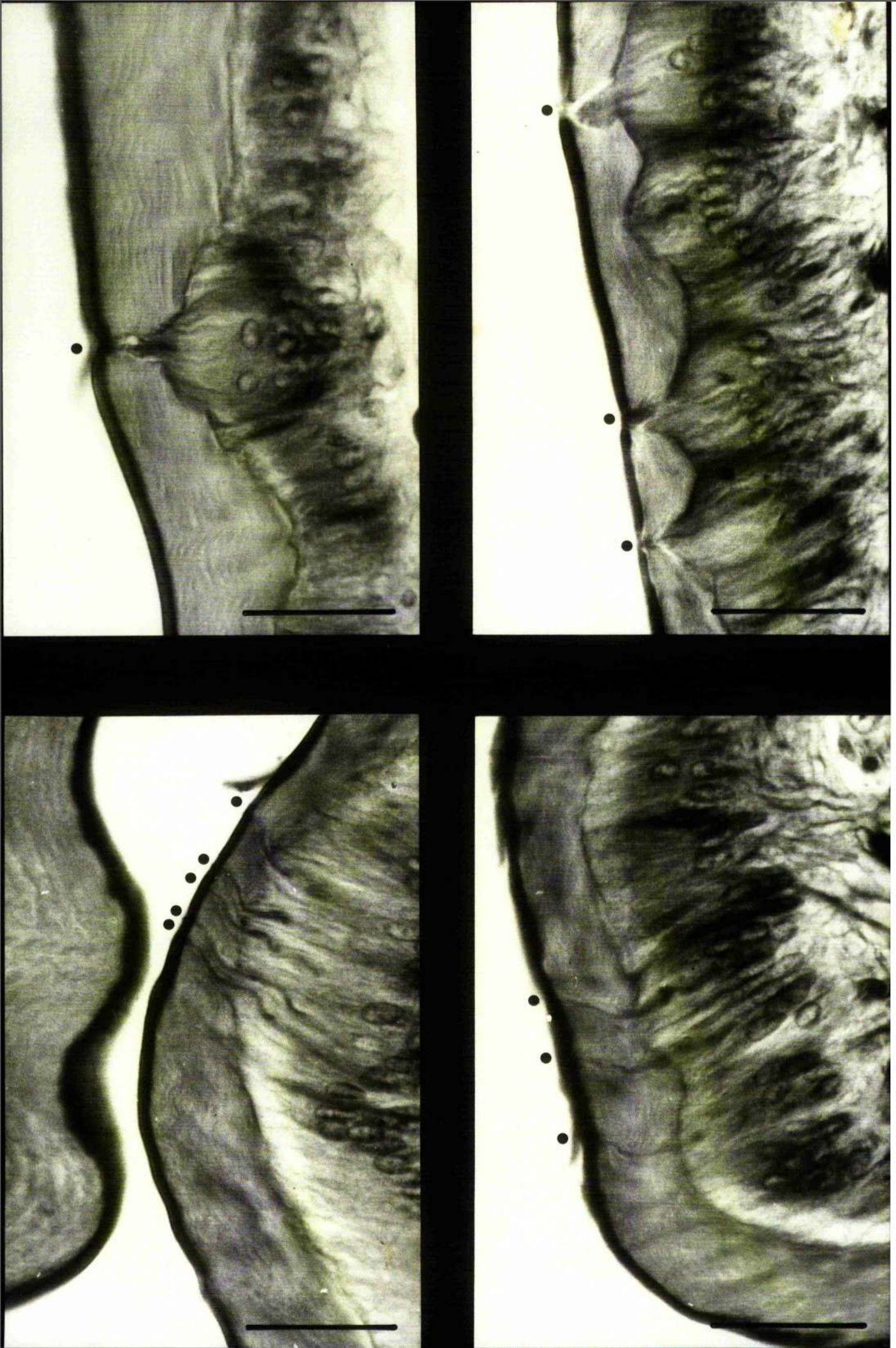


Figure 26

Block histogram showing the number and distribution of small and large pores (upper and lower graphs respectively) in the right A.C.S. The inset (circled) shows the direction of sectioning with regard to the whole organ, and the thickness of the sections.

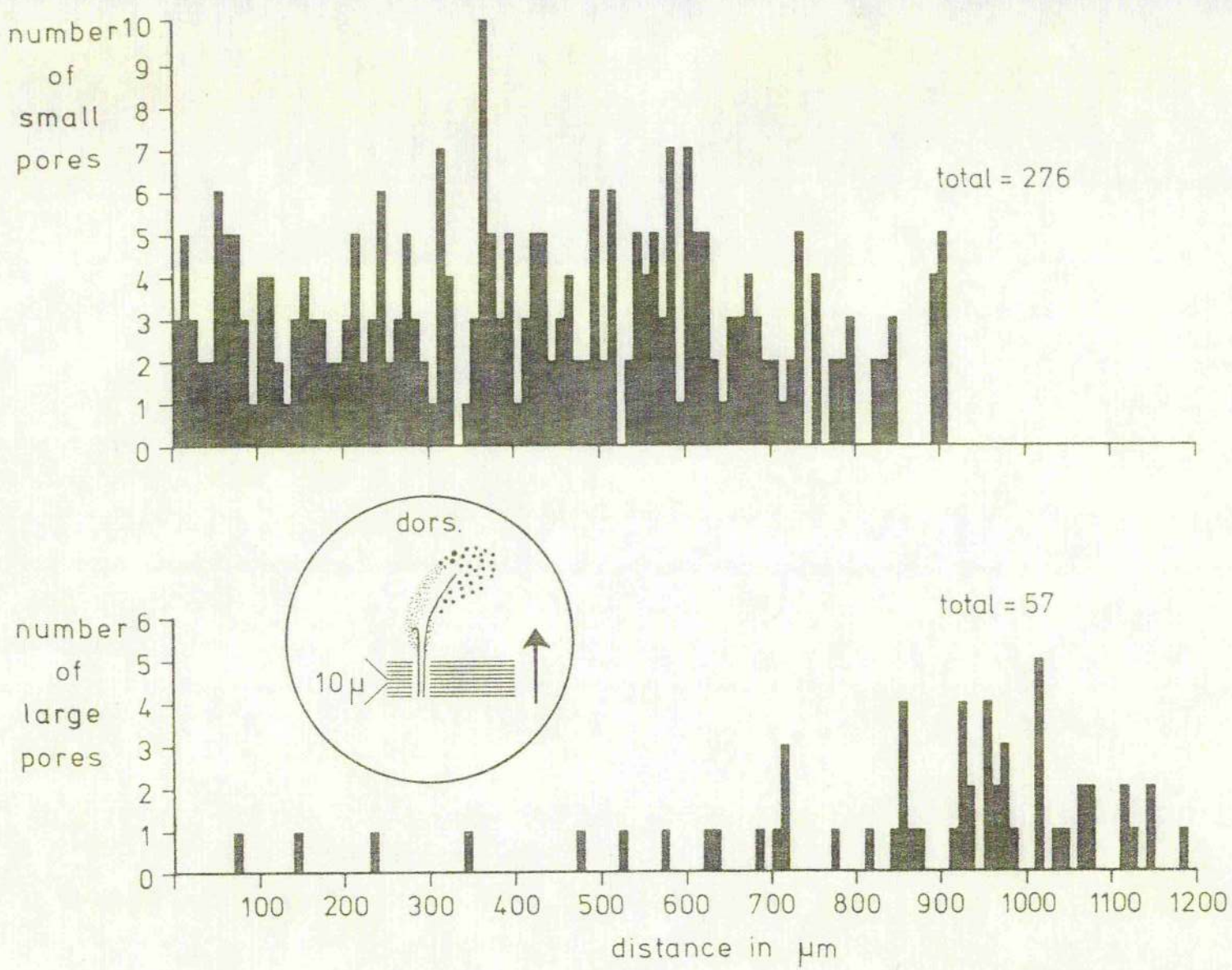

number of small pores
0 1 2 3 4 5 6 7 8 9 10
total = 276
dors.
10 µ
number of large pores
0 1 2 3 4 5 6
total = 57
100 200 300 400 500 600 700 800 900 1000 1100 1200
distance in µm

Figure 27

Scanning electron micrographs of cuticular structures associated with the left (a) and right (b) A.C.S. Note numerous hillocks with small depressions over the lateral walls of the anterior lobe of the C.C.S.V.

Scale mark = 200μm.

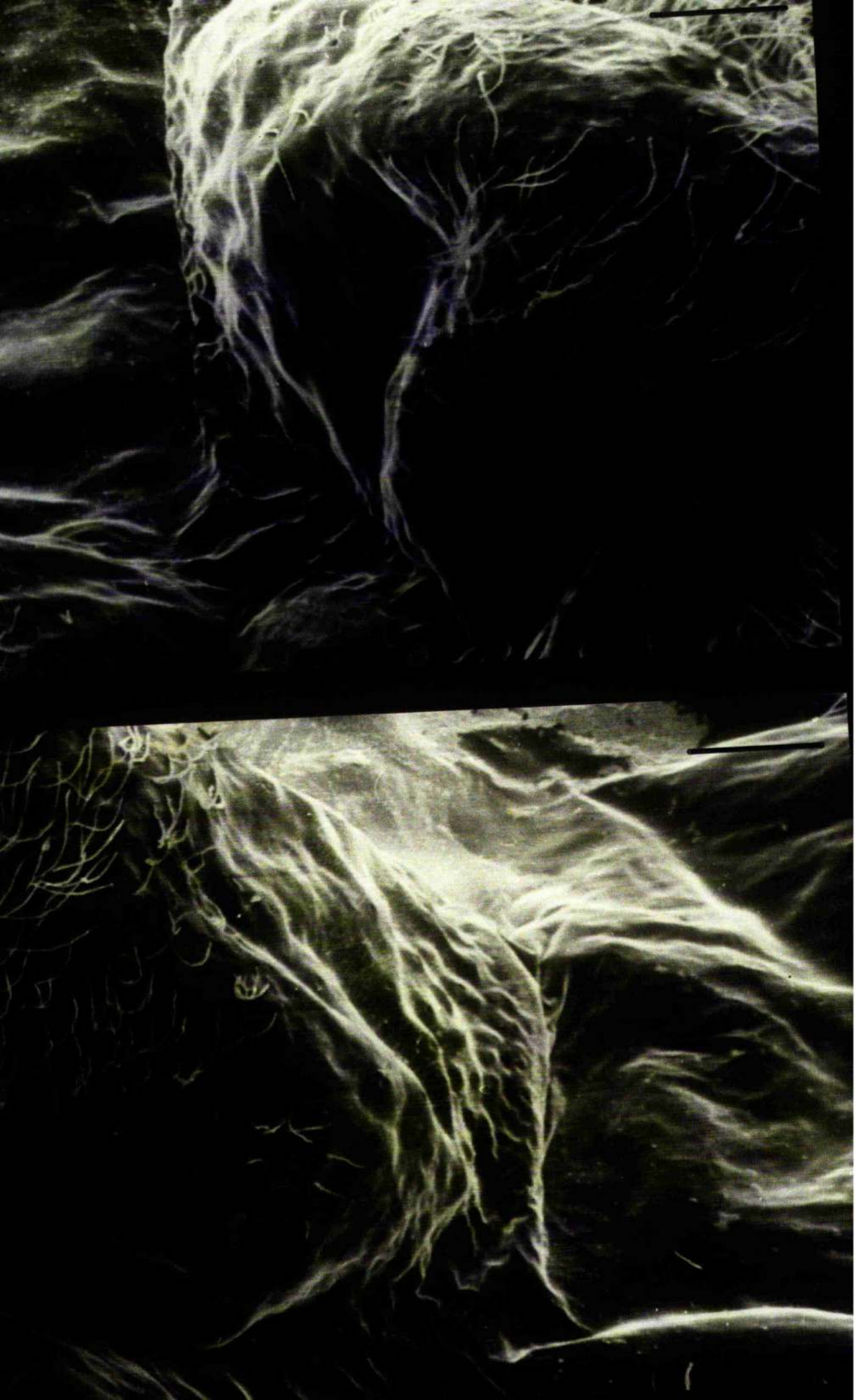


Figure 28

Scanning electron micrographs of cuticular structures associated with the A.C.S. - Large hillocks with depressions on their raised surfaces. Each depression contains a small number (1-4) of small nodules arranged in groups. There is a sparse covering of bristles between the hillocks, but the hillocks themselves are devoid of hair. Arrowed in b is a depression in the centre of a nodule. This may indicate the presence of a pore or a region of thin cuticle.

Scale mark - a: 40μm

b: 10μm

Figure 29

Scanning electron micrographs of cuticular structures associated with the A.C.S. - Small nodules (some indicated by dots) on the surface of the epicuticle. These lie in a narrow strip beside a prominent band of bristles. Arrowed in b is a depression in the centre of a nodule. This may indicate the presence of a pore or a region of thin cuticle.

Scale mark - a: 40μm

b: 10μm

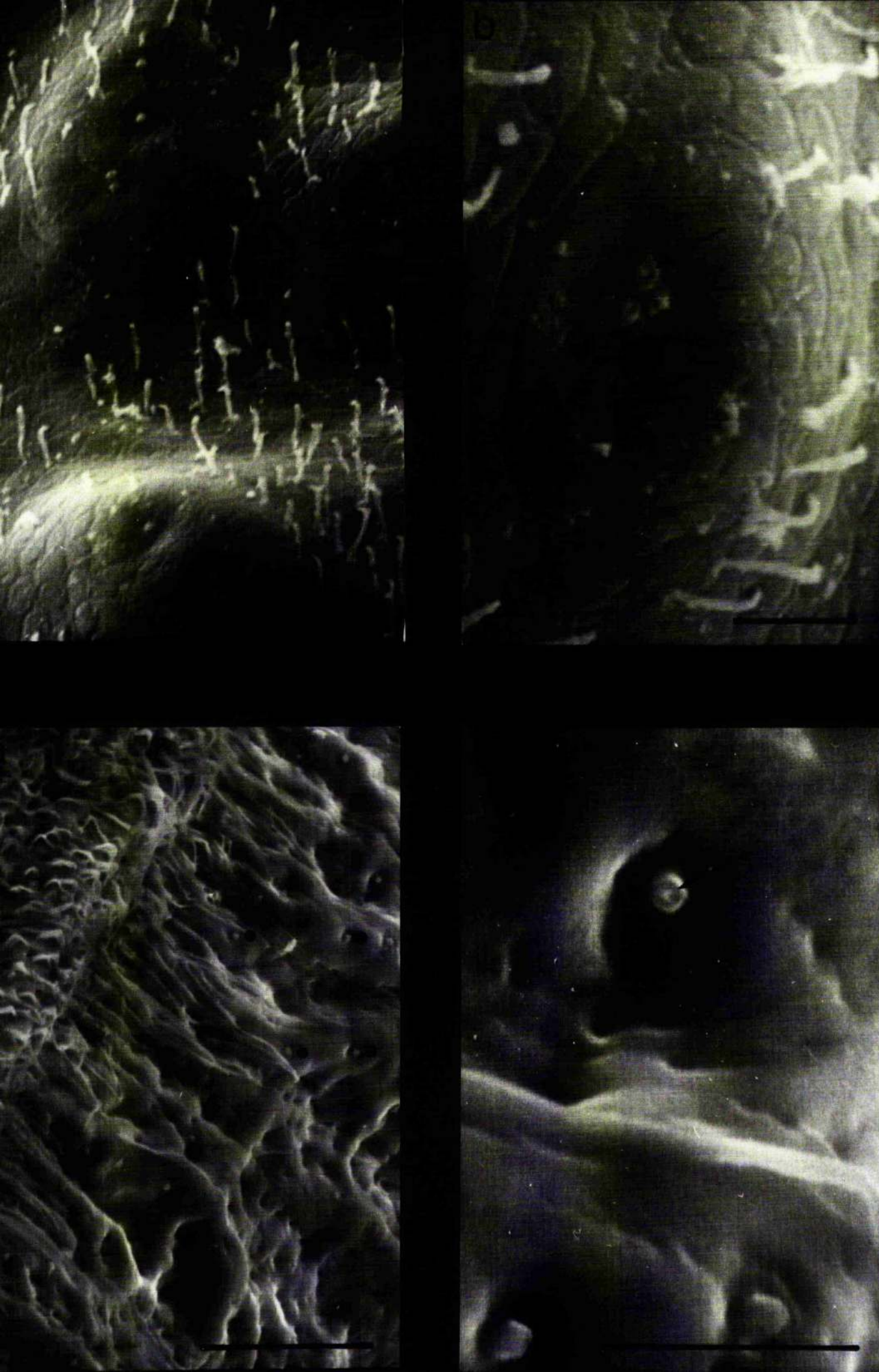


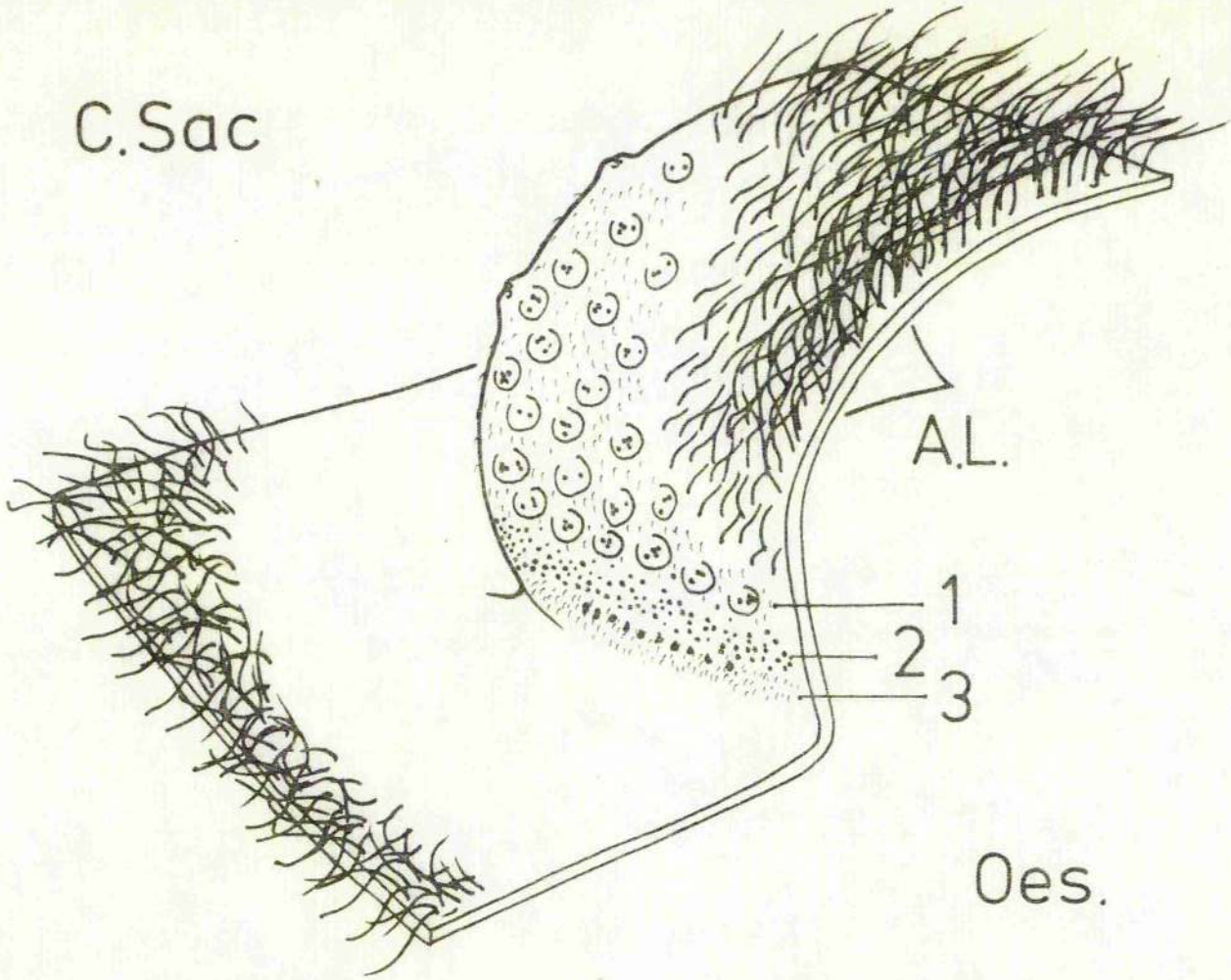
Figure 30

Ventro-lateral aspect of the left lateral wall of A.L. to show the three areas of structural modifications of the cuticular surface of the oesophagus in the region of the A.O.S.

1. Large hillocks with groups of small nodules in depressions
2. Narrow area devoid of hair but covered in small nodules
3. Band of bristles at the lateral limit of the A.O.S., commonly having a single line of pores at its medial edge.

C.Sac - cardiac sac

Oes. - oesophagus


C.Sac
A.L.
1
2
3
Oes.

Posterior Oesophageal Sensors

The posterior oesophageal sensors are to be found on either side of the posterior midline of the oesophagus at the entrance to the cardiac sac. They are present between the small posterior lobe and the lateral lobes of the O.C.C.V. and their positions are symmetrical about the midline.

(a) Methylene blue

The right P.O.S. is portrayed diagrammatically in Fig. 31, and photographs of Me. blue preparations are shown in Fig. 32. Each sensor is composed of 1 large group of sensory cells (150–200) and 2 or 3 smaller groups (50–70). Unlike the A.O.S., the groups of the P.O.S. do not appear to have a constant uniform shape in different animals, and their positions are variable within a limited area. The cells are small (10–15µm long axis of cell body), bipolar and uniterminal with short dendrites which terminate at the epicuticle. Their sensory axons travel in the v.-p.o.n. to the commissural ganglion (see Figs. 18, 20 and 21).

Viewing Me. blue preparations of the sensor with Nomarski-interference-contrast illumination shows that the dendritic endings are associated with distinct epicuticular structures which occur in an area devoid of large hairs but invested with a patchy covering of bristles. These structures were examined using the scanning electron microscope.

(b) Scanning Electron Microscopy

The epicuticular surface of the oesophagus in the posterior region of the O.C.C.V. is similar to that in the anterior region in that there is a large number of large hairs (200–500µm long and 6–10µm basal diameter). There are, however, no hairs in the area innervated by the P.O.S. Within this area distinct groups of epicuticular modifications can be seen. Fig. 33 shows one such group and two types of structure are noticeable:-

Figure 31

The posterior oesophageal sensor, posterior aspect

L.L. - lateral lobe of O.C.S.V.

O4 - oesophageal constrictor

O5a - dorsal limit of posterior oesophageal dilator

P.L. - posterior lobe of O.C.S.V.

P.O.S. - posterior oesophageal sensor

v-pon - ventral-posterior oesophageal nerve

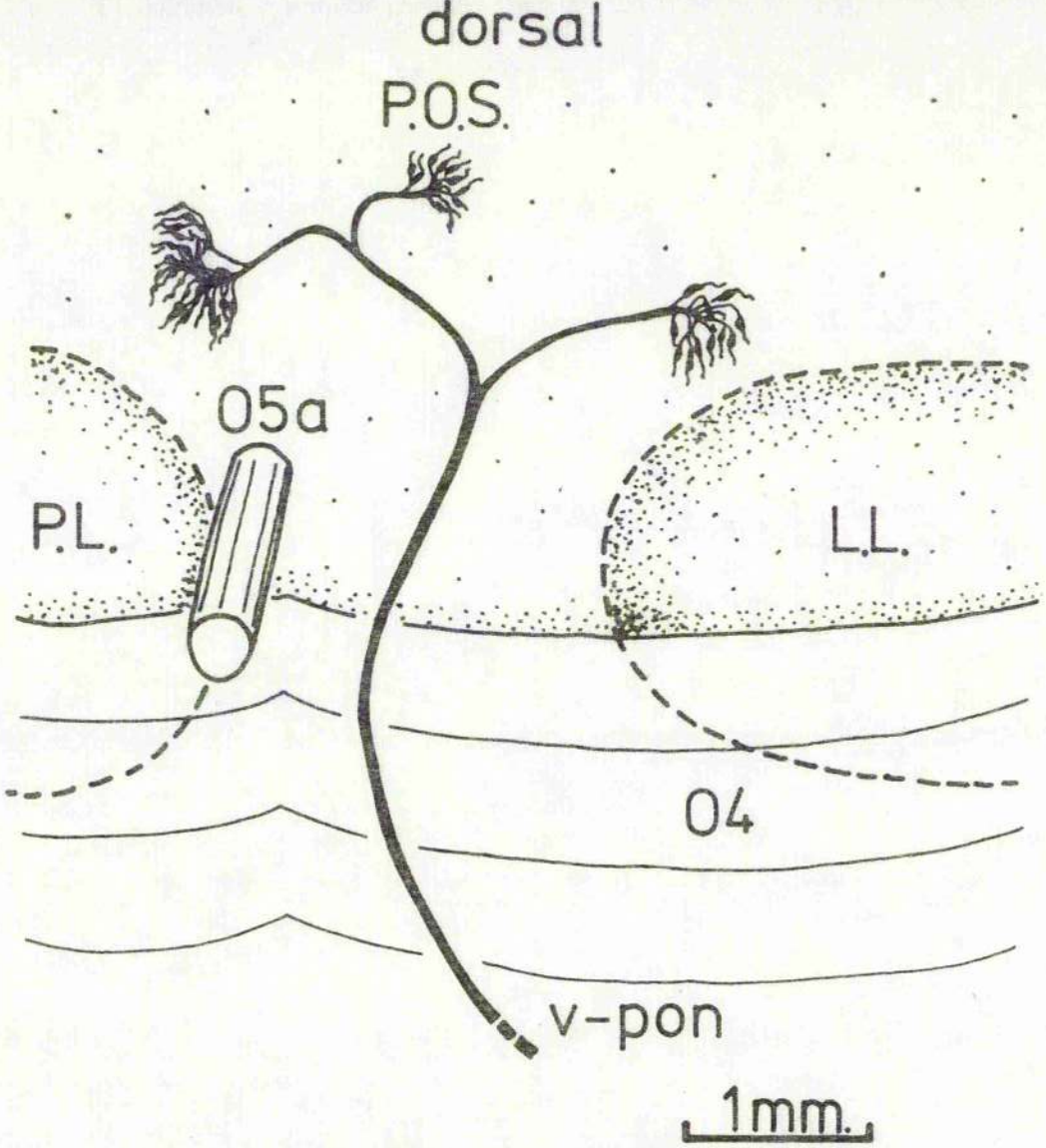

dorsal
P.O.S.
05a
P.L.
L.L.
04
v-pon
1mm.

Figure 12

Methylene blue staining of the P.O.S. a and b are photographs of parts of a P.O.S. in different animals, to show individual variation.

Scale marks - 200μm

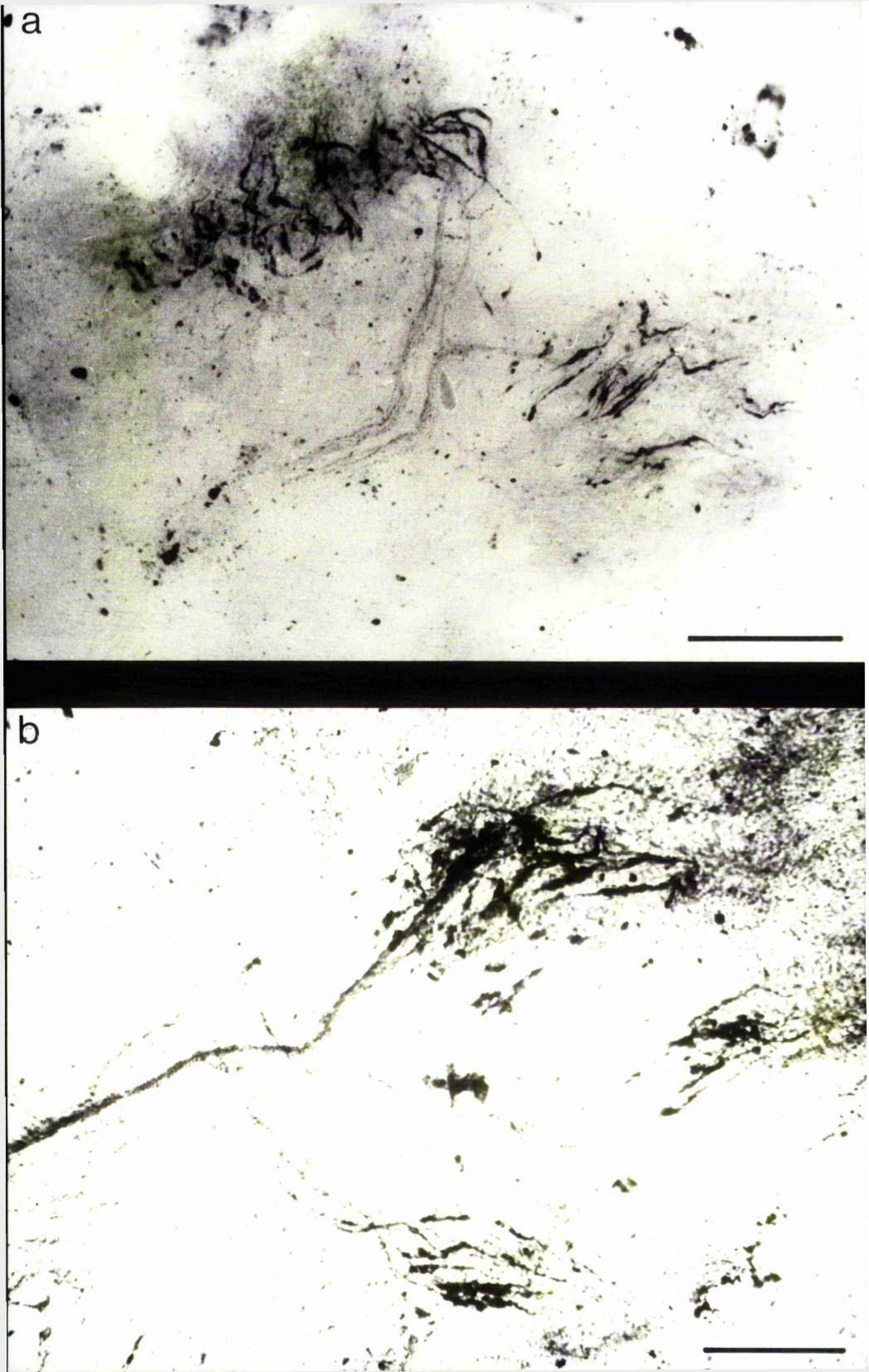

a
b

Figure 33

Scanning electron micrographs of cuticular structures associated with the P.O.S. - one small group of the P.O.S. to show a scattering of c.50 depressions and a patchy covering of bristles in a well defined area.

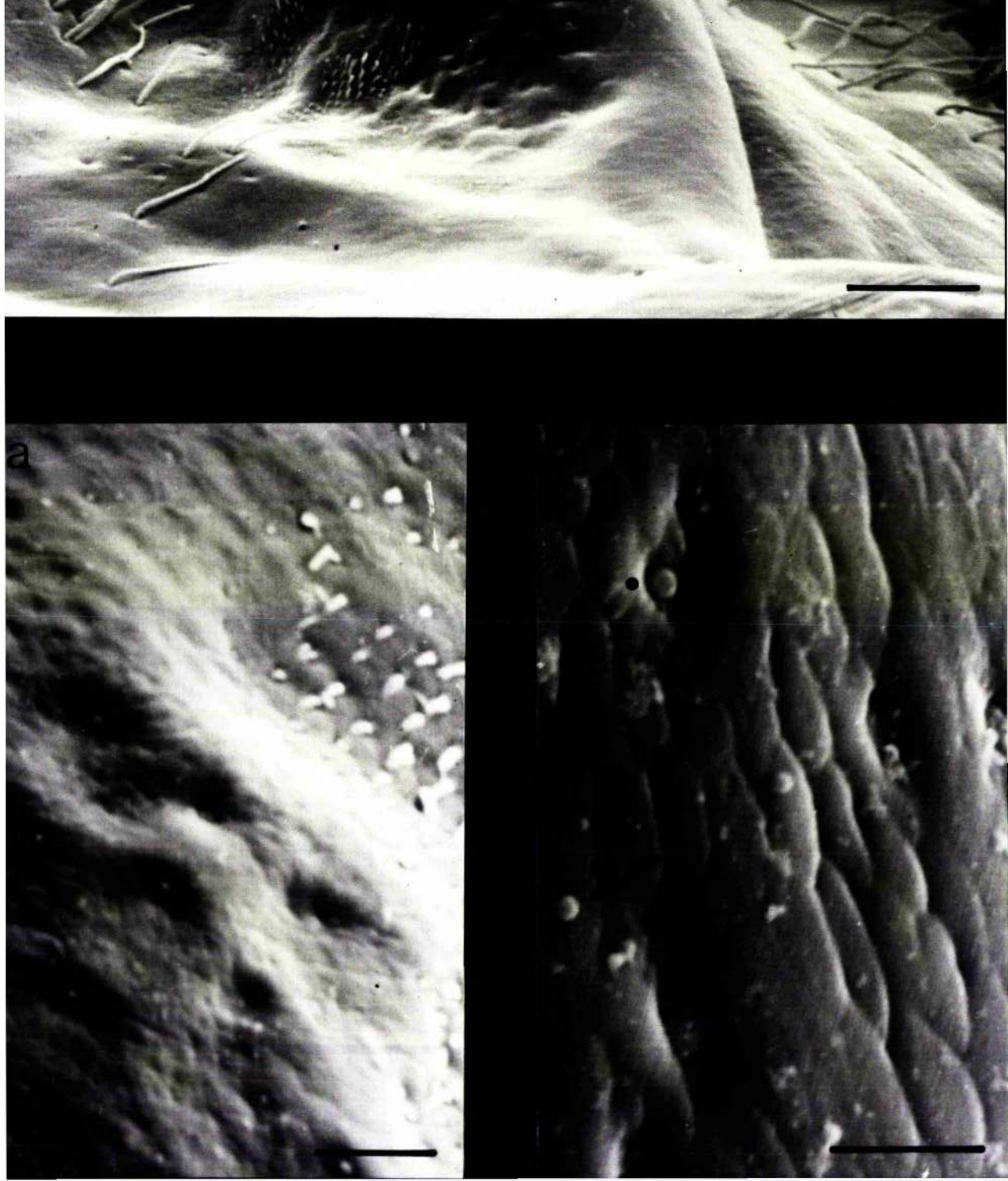
Scale mark - 100μm

Figure 34

Scanning electron micrographs of cuticular structures associated with the P.O.S. - a and b are close-ups to show the depressions in the epicuticle and the small spherical nodules in their centres (some indicated by dots). Arrowed in b is a nodule not confined to a depression.

Scale marks - a - 20μm

b - 10μm


a

(a) collections of small bristles similar to those described at the epicuticular surface of the A.C.F.; (b) a scattering of small depressions of the epicuticle. The latter are about 10µm in diameter, and each contains a small spherical nodule in its centre (Fig. 34a and b). These are approximately 2µm in diameter. Occasionally nodules are seen which are not associated with a depression (Fig. 34b arrowed) but this is seldom. The patterning of the bristles and depressions does not form any recognisable configuration between animals, save that the two are mutually exclusive.

4. DISCUSSION

Observations of the labrum

No fault can be found with the description of the labrum as a shield-shaped lobe overhanging the mouth. This is reflected in the name given to its main supporting element - the scutiform sclerite. The other components of the skeleton play a lesser part in maintaining the shape of the labrum and serve as the sites of muscle insertions and as suspensorial structures. The mouth is oval with its longitudinal axis in the anterior/posterior plane. The wide apron of thin cuticle which joins the anterior edge of the scutiform sclerite to the supra-labral ridge will ensure that the labrum can travel a relatively long distance between its anterior and posterior limits. Also, the skeleton of the labrum is nowhere rigidly connected to the rest of the animal but is merely suspended between the mandibles by the falciform sclerites. In part these will act as guides over the inner rims of the mandibles to control labral retraction and protraction. Thus the labrum can completely occlude the mouth and by a long retraction allow access to the oesophagus.

Several points about the labral structure and musculature are worthy of mention when considering its role as a moveable guard for the mouth. Although it is dangerous to try to define the functions of muscles from purely

anatomical data, it can be helpful to formulate a model of their possible co-operative action for two reasons: firstly, as an aid in elucidating the possible role of the whole structure; and secondly, to provide a basis for the development of future experiments. It is pointless and possibly harmful to wait until all is known before making the first tentative conclusions. It must be remembered that, because of the attachment of the labrum to the anterior oesophageal rim by the furcular sclerite, gross labral movements cannot be divorced from mouth opening and closing. There are only two joints in the labral skeleton. These are the complex bilateral attachments between the falciform, furcular, nodular and scutiform sclerites (Fig. 3) and they will play an important part in the repertory of labral movements by acting as pivots. With two exceptions (L1, O4a), the extrinsic and intrinsic muscles are orientated in such a way as to either retract the labrum, rotate it about the joints or shorten it and its lobe. All three of these modes of action could be used to effect mouth opening. For example, O1a, L4 and L5 will pull the labrum and the anterior edge of the oesophagus towards the supra-labral ridge (the posterior oesophageal rim is firmly attached to the metastomal plate); the bundles of L6 will cause the apron of cuticle to pucker inwards and will remove the lobe from the mouth area by inwardly rotating the anterior edge of the scutiform sclerite; and L2, L3 and L8 will reduce the lobe and shorten the labrum by folding it about a transverse axis. The flexibility of the labrum will be important in allowing it to be reduced in volume and crumpled against the supra-labral ridge. Closure of the mouth will be effected by the elasticity of its structure and the contraction of O4, the oesophageal constrictor. Of particular interest is L2. This muscle would appear to be capable of different actions depending upon the state of the mouth at the time of its contraction. With the mouth open and the labrum retracted, L2 will have the effect of shortening the lobe. However, if the mouth is closed and the oesophagus constricted by O4, then L2 will pivot the scutiform sclerite about its joints to

protract the labrum. This would aid the probable actions of L7 and O4a. Unaccounted for in the above resume is L1. The action of this muscle will undoubtedly be to medio-laterally constrict the labrum, making it narrower, but the reason for this remains mysterious. It is unlikely that it will play a part in labral retraction, as altering the shape of the labrum in that way would militate against effective retraction. The alternative, that it plays a part in protraction, is more attractive. One might speculate that as the lumen of the mouth is itself narrow then labral protraction would be helped by a narrowing of the labrum. It is also possible that contraction of L1 could blow out the lobe, or stiffen it, hydrostatically (Balch, personal communication). Further discussion concerning the labral musculature will be found in the next chapter where its active role in feeding is considered.

Comparison of this description of labral structure with others must be limited by the paucity of reports on this subject. Also, care must be taken when comparing these results with those obtained without the benefit of modern dissection microscopes (Lemoine, 1868; Mocquard, 1883; and Ringel, 1924). However, of the five muscles which have been described in the crayfish, two can positively be identified in the lobster. These are the transverse muscle of the labrum (L1), and the labral levator (L6). The others are retractor muscles (internal, external and medial) and it is of little value to try to compare these with those of the present description. The labrum of insects is better described and it is a much simpler structure (Snodgrass, 1935 and 1952). Although it varies throughout the insects it remains a moveable preoral lobe of the head. The musculature consists of a paired (or single) median compressor which in the majority of insects runs sagitally (in the cockroach they become transverse), and two pairs of long extrinsic muscles (the anterior and posterior labral muscles). In fact the labrum of Homarus compares more favourably in its complexity and potential for finely controlled movements with the insect hypopharynx. This may reflect the importance of these structures in feeding.

Sensory Systems of the Labrum

Interest in the sensory functions of the labrum began with the studies of Lemoine (1868) and Herrick (1895). These authors likened the labrum to a vertebrate tongue and considered it as the seat of the sense of taste. Herrick's tentative proposal that the tegumental glands performed this function was refuted by Yonge (1924) who suggested that they might be involved in chitin production. Since then a role in mucus production has been ascribed to these glands (Barker and Gibson, 1977). Dando (1969) has described small uniterminal sense cells in the labrum and he presumed them to be chemoreceptive. However, this cannot be confirmed by the present study. Methylene blue staining did not reveal any similar cells and no cuticular modifications which might be associated with chemoreceptor endings could be found with the S.E.M. Lemoine stated that behavioural responses could be obtained by applying salt, pepper, tobacco, vinegar and ammonia to the labrum. These results must be called into question because of the difficulty of applying the stimulating compound directly to so small a structure as the labrum without contaminating neighbouring organs. Chemoreceptive responses have been described from the chelae of the pereiopods which grasp food material, and from the 3rd maxillipeds which cut and manipulate food material (Shelton and Laverack, 1970). Thomas (1970) describes a variety of setae on the mouthparts and labrum of Austropotamobius pallipes, a crayfish, and postulates that some will be involved in chemoreception (the labrum of adult Homarus bears no setae). There are also presumptive chemoreceptors in the oesophagus (see below). In fact it is the proximity of these other organs which indicates that a chemosensory role for the labrum might be redundant. Also the advantage of testing food just as it enters the mouth, when it has already been tested by receptors on the mouthparts, is dubious.

The large bipolar cells of the labrum (Dando, 1969) can be divided into three bilateral groups; innervating the floor, the lobe and the lateral walls. They have an appearance similar to that of the abdominal cutaneous mechano-receptors described in the crayfish, Procambarus clarkii (Pabst and Kennedy, 1967). In this case the dendrites of individual bipolar and tripolar neurones (50-70µm in length) branch extensively in the hypodermis of soft cuticle. The mechano-receptors of the labrum compare well with this description although details of their association with the hypodermis are not available. Their distribution is such that most of the surface of the labrum is innervated. Although the labral floor, the scutiform sclerite, is not exactly soft cuticle, it is capable of deformation in any plane. The labrum will be deformed by food being pushed into the mouth and by any active part it may play in feeding. These receptors are ideally situated to monitor such deformations. The lobe is the most vulnerable area and while the number of cells innervating it is not larger than for the other groups, the branching pattern of their dendrites is more extensive. The larger number of cells in group c. (innervating the side walls) may indicate that more detailed information concerning the point of stimulation is required for this area. The responses of the labrum to mechanical deformation are investigated elsewhere (Chapter 4).

Observations of the Oesophagus

The general form and musculature of the oesophagus of Homarus differs little from the descriptions for other decapod crustaceans: Astacus (Hocquard, 1883); Cancer (Pearson, 1908); Nephrops (Yonge, 1924); and Jasus (Paterson, 1968). The anterior, lateral and posterior oesophageal dilators are all present and their courses are similar. However, it proved useful in this study to treat the oesophageal/cardiac sac valve (O.C.S.V.) as a separate entity with its own dilators. This concept arose after following

the fibres of these dilators through the oesophageal constrictor to their insertionson the cuticle. The anterior and lateral lobes of the C.C.C.V. are well developed and the muscles under consideration (CCCV1, 2 and 3) were found to be associated with them rather than with either the oesophagus or the cardiac sac. Whether this morphological differentiation mirrors a functional division or not, remains to be seen. A muscle equivalent in size, shape and position to CCCV1 has been described as an oesophageal elevator (Nocquard, 1883). While it will undoubtedly have this effect, its prime role must be to dilate the C.C.C.V. CCCV2 is a narrow, somewhat frail muscle which has not yet been described in other animals. CCCV3 is probably represented by the antero-lateral dilators of the foregut in Cancer (Pearson, 1908) and in Jasus (Paterson, 1968). In these animals it is classified as a foregut muscle rather than an oesophageal muscle and apparently inserts on the cardiac sac. It is also portrayed unnamed in Fig. 8 of Maynard and Dando (1974) for Homarus americanus, where its course is the same as in H. gammarus. In fact, with the exception of CCCV2 which appears to be lacking in H. americanus, the oesophageal musculature of H. Americanus as shown in that figure is a replica of that of H. gammarus.

The oesophageal constrictor is a complex muscle and little is known of its detailed morphology. Its ligamentous and muscular attachments to the oesophagus and surrounds are reported in this study and are of some interest. The presence of a separate band of muscle at the dorsal edge of C4 lends credence to the concept of a separate muscular system at the valve. Although, once again, the question of whether it is a functional division would have to be resolved electrophysiologically. This band has distinct attachments at the insertion of CCCV3a in the lateral lobe. The ventral muscular attachments to the paragnathal cuticle and to the furcular sclerite (C4a) may be significant in promoting effective mouth closure. The movement of the paragnaths

could prevent the loss of food material from the posterior part of the mouth as has been suggested by Farmer (1974) for Nephrops. O4a will pull the labrum over the mouth as it closes. This will be discussed further in Chapter 3 (labral movements during feeding). The ligamentous attachments are most likely simple anchorage points to prevent the ventral rim of O4 from riding up during contraction. The anterior limit of O4 is firmly attached to the cardiac sac by a large median antero-dorsal insertion. This will also aid food entry into the cardiac sac. Yonge (1924) and Barker and Gibson (1977) have described discrete bands of intrinsic longitudinal muscle beneath the constrictor. The bundles of longitudinal muscles which Barker and Gibson portray in their Fig. 2 lie outside the constrictor and are probably dilator bundles which were not cropped short enough before sectioning. Examination of the oesophagus in situ before sectioning would have made the interpretation of their sections more understandable. There are, however, distinct longitudinal bands anteriorly laterally and posteriorly where O4 is anchored to the oesophagus by the insertions of the dilators, and muscle fibres run longitudinally within these bands. Whether these longitudinal fibres are contributed by the dilators or by the constrictor would have to be discovered electrophysiologically due to the tangle in these areas.

This study also provides a map of the finer innervation of the oesophagus. It adds only a little to our present knowledge and is of little value, due to individual variation, save to show approximately whence the muscles are innervated. A better approach would be to determine the innervation pattern of the muscles electrophysiologically without regard for the specific nerves in which the axons may travel.

Sensory Systems of the Oesophagus

Large multiterminal neurons have been described innervating the oesophagus of larval Homarus (Allen, 1894) and Astacus (Allen, 1894, Orlov, 1926a,b). Dando and Maynard (1974) were unable to find large numbers of these

cells (as described by Allen) in Panulirus or Homarus but could confirm the presence of small numbers innervating the gut. In this thesis a small group of neurons with a relatively constant position is described. They are innervated by the a.o.c.n. and their dendrites travel over the oesophagus and cardiac sac in the region of the O.C.S.V. Allen described some cells as uniterminal but doubt was cast on this by Orlov. The dendrites of the cells described here are unbranched as far as they could be traced but this is not conclusive evidence that they are uniterminal. These cells are probably mechanoreceptors responding to stretch and are suitably placed to monitor movements of the O.C.S.V. They were not studied in detail as it was considered more useful to concentrate on those organs which are more amenable to physiological analysis due to their size and constancy of position. These are the oesophageal sensors (O.S., comprising the A.O.S. and the P.O.S.).

The O.S. are present in a ring around the oesophagus at the level of the O.C.S.V. Although the organisation of the A.O.S. is considerably more complex than that of the P.O.S., the individual receptor elements are the same in both organs. That is to say: a small (15-20µm long) bipolar neuron with a uniterminal dendrite which passes through the chitin layer and is associated with a small cuticular nodule located in a depression. In group a of the A.O.S. and in the P.O.S., these elements occur singly. However, in group b of the A.O.S. the elements are organised into bundles of 3-5. This can be seen both with light microscopy of Ma. blue stained preparations of the neurones and with an S.E.M. examination of their cuticular endings. In the latter case the nodules are grouped into the central depression of a relatively large hillock (50µm basal diameter). Mouline (1968) gave 5 morphological criteria for classifying organs as contact chemoreceptors. These are:

1. The similarity with previously described organs. Although similar organs have not been described in crustacean decapods, the O.S. are comparable with the epipharyngeal and hypopharyngeal organs of the cockroach, Blabera craniifer (Koulins, 1968, 1971), and with the A1 sensilla on the clypeo-labrum of Locusta migratoria migratorioides (Cook, 1972). The former have indirectly been shown to respond to the application of chemicals (Koulins, 1971). In these cases each "cone" (equivalent in size and shape to the "nodules" described for the O.S.) has been shown by transmission electron microscopy (T.E.M.) to be innervated by 4-5 bipolar neurons. In the O.S. the number of sense cells stained with Me. blue approximates the number of nodules seen with the S.E.M: but Me. blue staining is known to be capricious and only a proportion of the cells present probably will stain. T.E.M. is necessary before a definite statement about the number of cells innervating each nodule can be given.

2. Direct contact of the dendrite with the external medium. The presence or absence of a pore through the cuticle at the apex of the nodule is a debatable question. Cook (1972) could not identify pores in the A1 sensilla with the T.E.M., but she considered that small central depressions of the cones observed with the S.E.M. were indicative of pores. The nodules of the O.S. have similar depressions. However it is possible that these are regions of very thin cuticle which have collapsed during the drying process. Other well described crustacean chemoreceptors, the antennular aesthetasc hairs (Laverack and Ardill, 1965) have no pores (Laverack, 1975) and Chiradella, Case and Cronshaw, (1968b) claim that the permeability of the hair wall is sufficient to allow access of the stimulating substance to the dendrite. This may be the case for the O.S.

3. A lack of modification of the peripheral processes. Mechanoreceptors tend to contain dense material (the scolopale) surrounding numerous microtubules in the apical region (Thurm, 1965, 1968). For the C.S. this would need to be confirmed with a T.E.M. study.

4. A large number of sensory cells. Mechanoreceptors tend to have small numbers of neurons compared with chemoreceptors. The A.C.S. and P.C.S. comply with this criterion by having 400-600 and 250-350 neurons respectively.

5. The position of the organ in the animal. The C.S. are admirably situated to sample food material in the oesophagus.

The C.S. are thus classified as contact chemoreceptors on morphological grounds. Indirect evidence that they respond to the application of a food extract will be given in Chapter 4.

Little can be said about the overall structure and position of the P.C.S. which occurs as amorphous groups. However an important point to note concerning the A.C.S. is that its endings are situated deep in the cleft between the anterior and lateral lobes of the C.C.C.V. In their normal position these lobes will completely occlude the organ; the bare patch of cuticle on the lateral lobe covering the cuticular structures on the anterior lobe (Fig. 30). The disposition of the CCV dilators (1 and 2) is such that their contraction will pull the anterior lobe forward, as a whole, without collapsing it. This is not the case for the lateral lobes which have the insertions of CCV2 (right and left) deep in their cavities. Thus, during the normal opening and closing of the valve, the A.C.S. will not be available for stimulation and it will only become so when the cardiac sac is filled to capacity and the C.C.C.V. is stretched open. Possible reasons for this will be discussed in Chapter 4.

Similar organs to the A.C.O. have been described in Astacus and Panulirus although the arrangement in Panulirus is somewhat different (Dando and Maynard, 1976). Orlov (1926a,b) described small uniterminal cells scattered over the oesophagus and numbering about 300, and Hingel (1964) described a series of sensory plates, invested with small cuticular pegs, on the surface of the ventral cardiac gutter. These may correspond with the A.C.O. described here in Homarus, although there are differences. It is possible that the fine structure of the endings of these organs are unique to gut chemoreceptors which are bathed in the stimulating medium and thus may not need the advantage of being situated on hairs. It is probable that comparative studies on other decapod crustaceans will reveal organs similar to the G.O.

CHAPTER 3

LABRAL MOVEMENTS DURING FEEDING

LABRAL MOVEMENTS DURING FEEDING

1. INTRODUCTION

The labrum of decapod crustaceans is a soft lobe overhanging the mouth, which possesses a complex musculature. Its medial position makes it an ideal structure to aid food entry into the oesophagus. It is therefore surprising that its role in the mechanics of feeding has not yet been investigated in detail.

Review of Labral Movements and Function

The dearth of information on labral function is such that most of the relevant references to it can be quoted verbatim.

1) "Between each bite, which is assisted by the tearing action of the second maxillipeds, the palps and labrum descend, pushing material up into the oesophagus and cleaning out the groove behind the cutting edge of the mandible." Nicol (1932) for Galathea dispersa.

2) "the labrum plays an active part in holding food or tucking it into the mouth." Marshall and Orr (1960) for crustaceans.

3) "The labrum .... (is) capable of various movements and appear(s) to be complementary to the mouthparts and the mandibular palps in holding the food between the bluntly tuberculated mandibles while it is being crushed prior to its passage into the oesophagus." Paterson (1968) for Jasus lalandi.

4) "The distal segments of the two (mandibular) palps, together with the labrum, form a very mobile anterior wall to the mandibular chamber, made more effective by the presence of the teasel setae." Thomas (1970) for Austropotamobius pallipes.

5) "The mandibular palp .... aided in the movement of food into the mouth, as did ... the labrum (the upper lip)." Caine (1974) for Cyalipes gundulpensis.

6) ".... the entrance of food into the gut .... is facilitated by dilation of the mouth and the alteration in position of the labrum." Laverack (1974a) for decapod crustaceans, especially the lobster.

7) "Both pairs of maxillae .... push the food into the gaping mouth below the raised labrum." Barker and Gibson (1977) for Homarus gammarus.

8) "The labrum is provided with muscles running largely fore and aft that enable it to deform its posterior face and thus, by movements akin to those of peristalsis, perhaps to aid movement of the food, though its part in this respect can be only minor." Fryer (1977) for Atyid prawns of Dominica.

Yonge (1924) also gives a brief description of the feeding mechanism of Nephrops norvegicus, without describing labral movements in detail. Farmer (1974) in his account of the feeding of Nephrops neglects to mention the labrum at all, but in this case the study was primarily concerned with the structure and function of mouthpart setae.

Apart from the above descriptive studies, the work of Laverack and Dando (1968), Moulins (1969) and Moulins, Dando and Laverack (1970) on the mouthpart receptors is of some relevance to a study of the function of the labrum. Using several decapod crustaceans (Homarus gammarus (1968), Panulirus argus, Nephrops norvegicus and Astacus leptodactylus (1970)) they describe the three mouthpart receptors, MPR1, 2 and 3, and characterise their responses to mandibular, labral, paragnathal, buccal and oesophageal movements. With reference to the labrum they state that "It is capable of diverse movements of large amplitude due to the activity of the intrinsic

musculature". Three types of labral movement during feeding are described: Type 1 - movements towards the mouth; Type 2 - movements away from the mouth; and Type 3 - shortenings of the labrum. When these three types of movement are imposed on the labrum, the activity of the mouthpart receptors is modulated in various ways. The conclusions are that minimal activity of MPR1 indicates a withdrawn position of the labrum (type 3) and increased activity occurs when the labrum is moved away from the rest position, either forwards or away from the mouth (type 1 and type 2). Also the MPR 2-3 system (considered as one receptor) shows more sensitivity to type 3 movements since it increases the impulse frequency of a tonic unit at slow speeds; recruits a position unit at fast speeds; and introduces a phasic unit at very fast speeds. Observations of the anatomy of the labral/oesophageal complex (see previous chapter) show that it is doubtful whether passively imposed shortenings of the labrum will not also move the mouth, and it is more likely that an active shortening of the labrum will have no effect on MPR 2-3. However, the information available on the movements and function of labrum can be summarised as follows: the labrum can undergo various types of movement and probably acts to facilitate food ingestion.

Object of Research

It is easily seen that little detail is known about the function of the labrum of decapod crustaceans, despite its prominent central position and complex anatomy. (The movements of the other mouthparts have been better described, and a description of the general feeding sequence will appear in the results section of this chapter). Also, there has recently been considerable interest in the control of foregut motility in decapod crustaceans (for a review see Selverston, Russell, Miller and King, 1976). It will add to this work to be able to characterise the sensory input to the various neuronal networks during normal feeding. The work of Moulins, Nando and

Laverack (1970) has gone a long way towards this end for the labrum. However, until more is known about the actual movements of the labrum during feeding little can be said about the amount and nature of the afferent input. For this reason, and to fill a gap in the knowledge of the mechanics of food ingestion, the aim of this project was to describe the movements and possible function of the labrum during feeding in the intact animal (Homarus gammarus). Unfortunately the proximity of the other mouthparts and the size of the labrum limited this to a study of the anterior/posterior (opening/closing) movements.

2. MATERIALS AND METHODS

A lobster which had been starved for about a week was strapped to a rigid perspex frame with elastic bands. A small hook, fashioned from an insect pin, was inserted into the scutiform sclerite of the labrum and the animal was then suspended in a perspex tank (38cm x 22cm x 20cm deep) which was supplied with a continuous flow of fresh sea water (Fig. 35). A length of thread ran from the hook and was attached to one end of the recording arm of a kymograph. The thread thus lay in a position vulnerable to interference from the chelae and the various mouth appendages. As a result, it was found necessary to restrain the chelae and remove the mandibular palps. In the latter case, the palps were excised at their joints with the mandibles, and the holes thus formed were plugged with small wads of tissue paper. These preparations were usually sufficient to enable relatively interference-free recordings to be obtained, although the 3rd maxillipeds did occasionally cause spurious movements of the recording arm.

After leaving the animal for about ½ hr. to become accustomed to its new situation, anterior-posterior (opening-closing) movements (Fig. 36) of the labrum were monitored. Two methods of recording these movements were

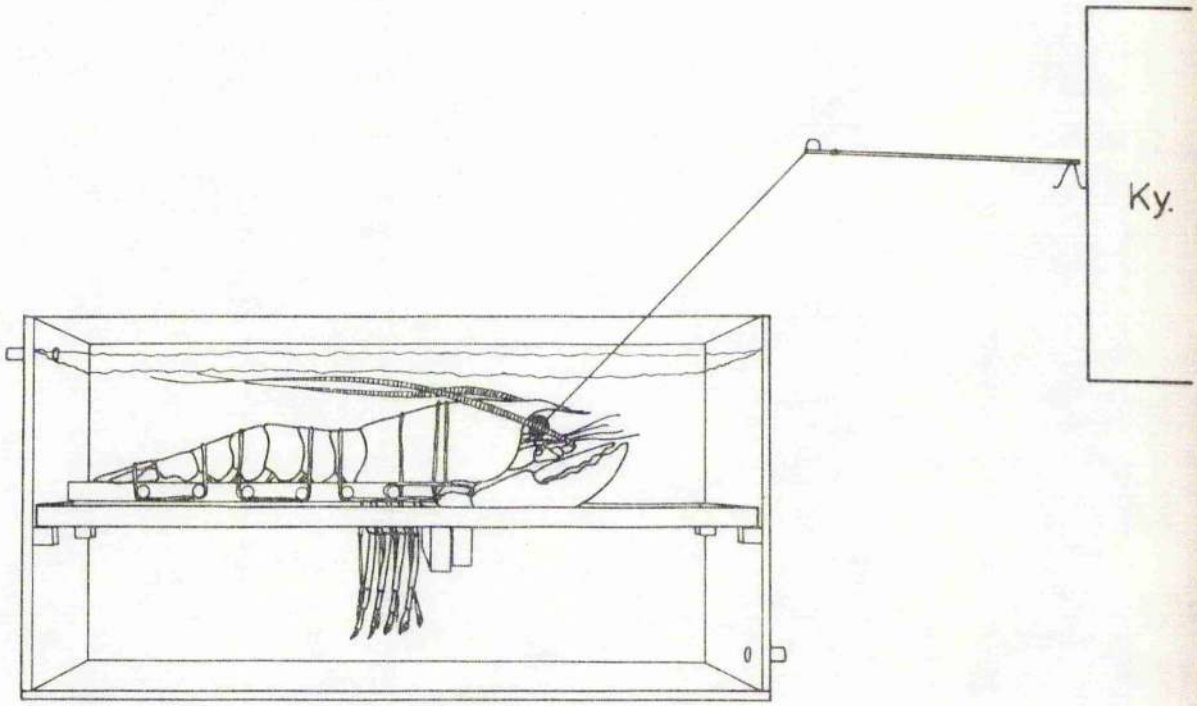
Figure 35

Set-up to record labral movements during feeding. A lobster is strapped to a rigid perspex frame and suspended in a tank supplied with a continuous flow of fresh sea water.

A - Side view with Kymograph (Ky.)

B - Top view with photocell (Ph.cell) recording system

A.



B.

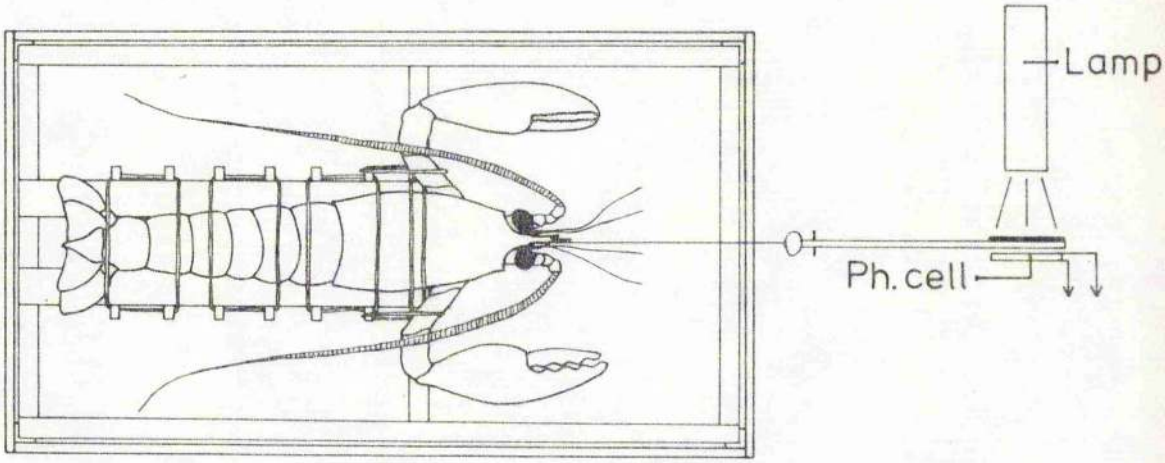
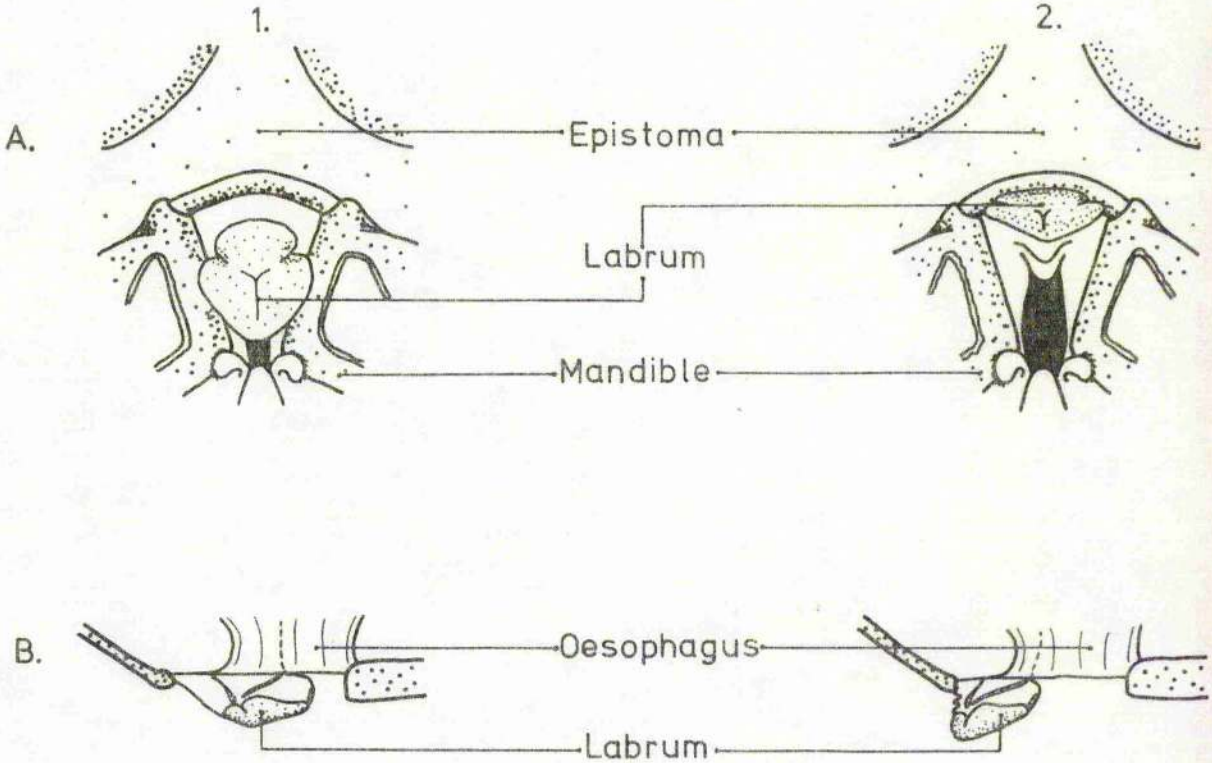


Figure 36

Antero-ventral (A) and left lateral (B) aspects of opening and closing movements of labrum.

1. Protracted (closed)
2. Retracted (open)


1.
2.
A.
Epistoma
Labrum
Mandible
B.
Oesophagus
Labrum

used. Firstly, to monitor gross movements, the recording arm was applied to the smoked surface of a kymograph drum rotating at fixed speeds. Secondly, in order to amplify the finer movements of the labrum, a photosensitive system was employed. A rectangular pennant of stiff, lightweight cardboard was affixed to the free end of the recording arm, and was positioned so as to interrupt a beam of light which illuminated a photocell transducer. Posterior (closing) movements caused an increase in the illumination of the photocell, whose output was amplified and recorded by a Devices hot-wire pen recorder (trace speed 1mm/sec.).

Using both recording systems, lobsters were fed with the legs of Nephrops norvegicus, small pieces of Mytilus edulis, and a length of rubber equivalent in size and shape to a Nephrops leg. As the chelae had been restrained, these items were proffered to, and readily accepted by, the 3rd maxillipeds. In an attempt to negate any effects of the unnatural visual environment, several experiments were performed on animals that had been blinded by covering their eyes with caps of aluminium foil. No difference in the labral movements under these conditions was apparent.

3. RESULTS

General Feeding Sequence

Observations on a tethered lobster indicate that feeding is accomplished in the following manner. Food material is either collected by the 3rd maxillipeds, or collected by the chelae and passed to the 3rd maxillipeds. The 2nd and 3rd maxillipeds manipulate the food into the proper orientation for eating, depending on its size and shape. Contact of the food with the inner mouthparts (1st and 2nd maxillae and 1st maxillipeds) and the rim of the mouth, causes the mouth to open by the abduction of the mandibles, the retraction of the labrum, and the dilatation of the most

ventral portion of the oesophagus. Small pieces of food are pushed into the opening provided and swallowed whole by the adduction of the mandibles, protraction of the labrum and initiation of peristalsis in the oesophagus. Larger pieces of food cannot be handled in this way, and must be broken down into pieces of a manageable size. This is performed by the co-operation of the mandibles and 3rd maxillipeds in biting actions. The food is manipulated by the mouthparts until a suitable amount is in the lumen of the oesophagus. The mandibles then close on the food, clamping it between their biting edges. The serrated edges of the 3rd maxillipeds bite just ventral to this point, and the 3rd maxillipeds pull away from the mandibles (Fig. 37). This usually has the effect of breaking, or tearing the food in two, and the part which is bitten off can be swallowed. If this is not the case, the sequence is repeated. During the biting phase the inner, manipulative mouthparts are drawn well out of the way, and between bites they recirculate the food.

After eating, and occasionally during the manipulative phases of eating, the exopodite flagella of the maxillipeds perform short sequences of rhythmical beating activity which set up water currents away from the mouth region. This is presumably to remove food debris from the area thus preventing contamination of the mouthparts, and the continued stimulation of chemoreceptory organs.

Biting on a Non-food Substrate

Figure 38(a) shows a pen-recorder trace of the opening and closing movements of the labrum during a biting sequence on a piece of rubber equivalent in size and shape to a Nephrops leg. A lobster will attempt to eat pieces of rubber if they are presented to the mouthparts in such a way as to stimulate them mechanically. For obvious reasons, the feeding response is more readily achieved if the rubber is first coated in homogenised Mytilus. At the beginning of the sequence the labrum retracts to allow the end of the

Figure 37

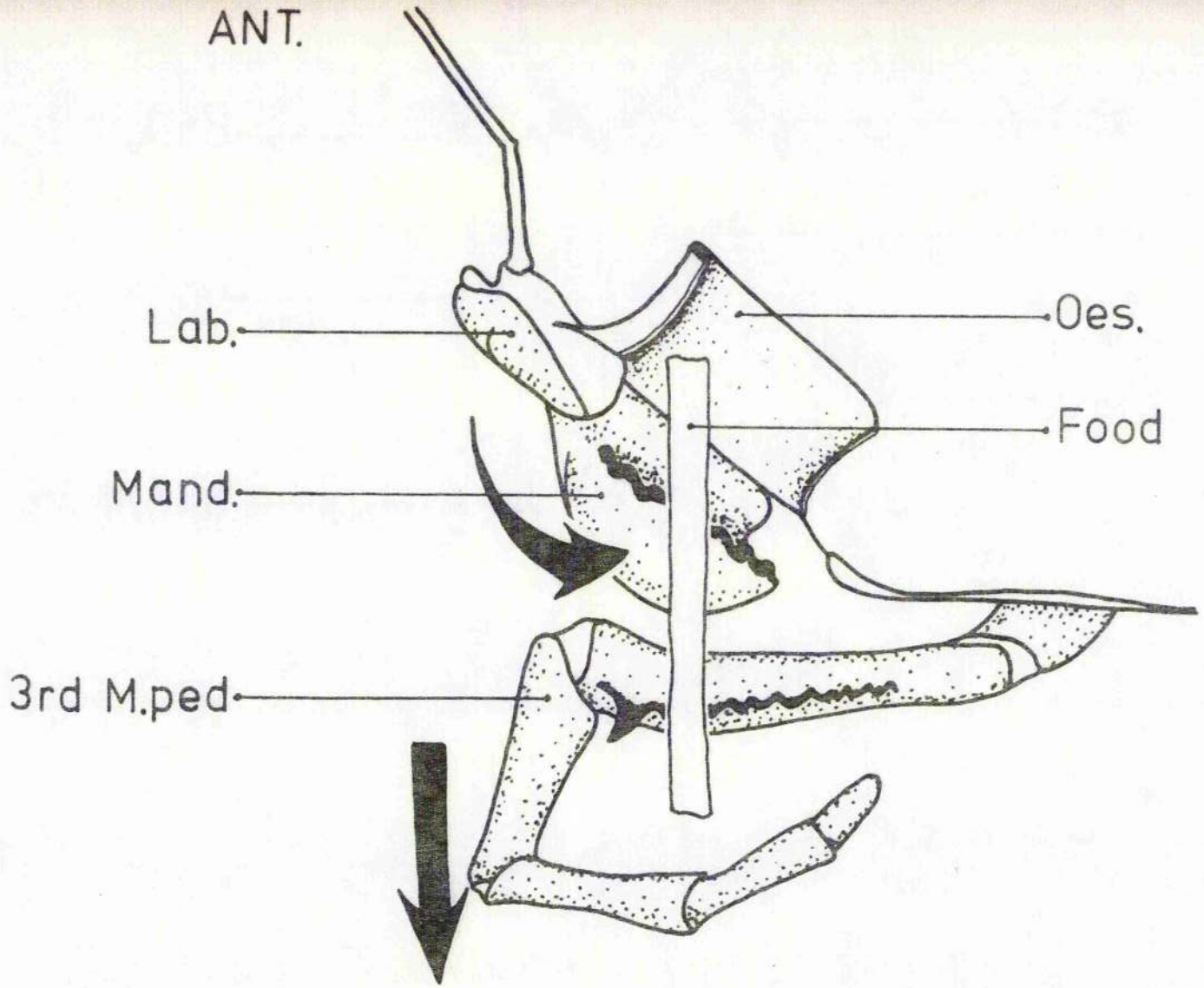
Co-operative action of mandibles and 3rd maxillipeds

Lab. - Labrum

Mand. - Mandible

3rd M.ped. - 3rd maxilliped

Oes. - oesophagus


ANT.
Lab.
Oes.
Food
Mand.
3rd M.ped

rubber access to the lumen of the oesophagus. Thereupon it closes as far as it is able, and between bites can be seen to retract a short distance to allow manipulation of the substrate if this is necessary (correlation between movements of the labrum and those of the other mouthparts was detected visually). Biting and manipulation are performed by the mouthparts as described above. It is doubtful whether the labrum exerts any pressure on the rubber when it closes during the biting phase. Each bite typically lasts 4-7 secs, and 10-15 biting cycles are usually sufficient to terminate the sequence before the rubber is discarded. The frequency of biting starts at about 0.2 cycles/sec (C/S) and slowly decays until the end of the sequence. After each successive bite the labrum retracts a little further, until it retracts fully to aid in discarding the rubber. The final closing movement of the labrum is substantially slower than the initial opening movement, and can take 10-15 secs. It can also be seen that the labrum does not close fully, having approximately 1mm more to close until the original resting position is attained. An important point to note is that after sequences when nothing is ingested, there are no further closing or opening movements of the labrum.

Ingestion of Food Material

(a) "Chewing"

When Nephrops legs or Mytilus pieces are being eaten, the opening and closing movements of the labrum are basically similar to those shown when the inedible rubber is chewed. With a Nephrops leg, biting and manipulative phases can be seen (Fig. 38(b)). The labrum lies near its resting position during a bite, and retracts between bites to allow the food to be repositioned. With pieces of mussel (Fig. 38(c) and (d)), the labrum simply retracts to let the inner mouthparts push the lump into the lumen of the oesophagus. Large pieces of mussel are torn apart by the same co-operative action of the mandibles and 3rd maxillipeds as previously described, and the labrum can be seen to

protract at each bite. The bites are of a short duration because the tissue is relatively flimsy, and minimal pressure is needed.

(b) "Swallowing"

After all the food has been broken down and pushed into the oesophagus, the labrum closes. This closure is more rapid than that observed when nothing is ingested (Fig. 38(a)). Also the extent of closure is initially greater than the original resting position which is regained gradually during the subsequent activity. Superimposed upon this can be seen small (0.5 - 1.0mm) rhythmical closing movements which decrease in size with time (Fig. 38(b)(c)(d) and Fig. 41). This activity is thought to be related to oesophageal peristalsis and will be termed "swallowing" activity. The cycles are typically biphasic, having a pause in protraction after c. 1 sec. before continued protraction and subsequent retraction to the heightened resting position. The whole cycle lasts approximately 2.5 secs. These small protractions can also be seen during the chewing sequence of feeding (Fig. 38(b)). Studies were made on the duration and frequency of this cyclical "swallowing" activity.

The duration of activity varied considerably between about 1min. to 35 mins. This was found to depend in some way on the amount of food which had already been consumed. Starved animals had short sequences of "swallowing" after a piece of food, fed animals had long sequences, and satiated animals tended to be erratic, stopping and restarting the sequence at irregular intervals. Occasionally a stringy piece of mussel became caught around the pin fixing the thread to the scutiform sclerite of the labrum. This resulted in food being present in the oesophagus, but being unable to move. In these cases, "swallowing" movements of low amplitude and at a low frequency continued until the food was freed, whereupon the frequency and amplitude of the movements would increase and then decay normally.

Instantaneous frequency plots of the cyclical activity in 4 short duration "swallowing" sequences are shown in Fig. 39. The frequency of a cycle is calculated as the reciprocal of the inter-cycle interval, which is measured from the start of that cycle to the start of the succeeding one. It can be seen that the initial frequency ranges between 0.3 and 0.5 C/S, and this fairly rapidly decays to just over 0.1 C/S. For longer sequences the initial frequency is lower (0.2 - 0.23 C/S) but the decay is not as rapid. This is shown in Fig. 40 which is a block histogram, for eight samples, of the number of cycles in successive 30 second bins. The larger standard error bars in the final minute are due to the cessation of cyclical movements in two of the samples after 4 mins. This would also necessarily reduce the mean values in the final minute.

A kymograph trace of the longest recorded activity is shown in Fig. 41, and this is graphically displayed in Fig. 42. It is noticeable in Fig. 42, (not depicted in Fig. 41), that feeding towards the end of cyclical activity will restimulate the system to a higher frequency. This frequency (0.23 C/S) is not as high as the original post-feeding frequency (0.26 C/S) and decays within a minute to a steady frequency of 0.15 C/S.

To summarise - "Swallowing" sequences in starved animals are of a short duration, with a high initial frequency which rapidly decays. "Swallowing" sequences in fed, but unsatiated, animals are of a longer duration, with a lower initial frequency which does not decay as quickly.

It is important to notice that the frequency seldom, if ever, dropped below 0.1 C/S without the subsequent cessation of movement. 0.1 C/S is thus to be regarded as the minimum possible frequency of swallowing activity.

Figure 38

Labral movements during feeding. A downward movement of the trace indicates retraction (opening) of the labrum.

(a) Chewing on a piece of rubber: Each bite of the mandibles is accompanied by a protraction of the labrum (arrowed). At the end of the trace the labrum retracts to aid in discarding the rubber. Note that the final closing movement is slow, that the original resting position is not attained, and that there are no further movements of the labrum.

(b) Ingestion of a Nephrops leg: A complex chewing (biting and manipulative) phase is followed by small protractions (swallowing) superimposed on a level of closure greater than the original resting level. Dots indicate where these small protractions can be seen during the chewing sequence.

(c) Ingestion of a small piece of Mytilus: Opening .... brief manipulation .... closing, is followed by a short sequence of swallowing.

(d) Ingestion of a large piece of Mytilus: (continuous trace) The details of the chewing phase cannot be seen as the labrum retracted out of the range of the recording system. Rapid closure is followed by prolonged swallowing. The level of closure during the swallowing sequence is c.2mm further than the resting level. This decays to the resting level within the subsequent 3 mins.

(further details in text)

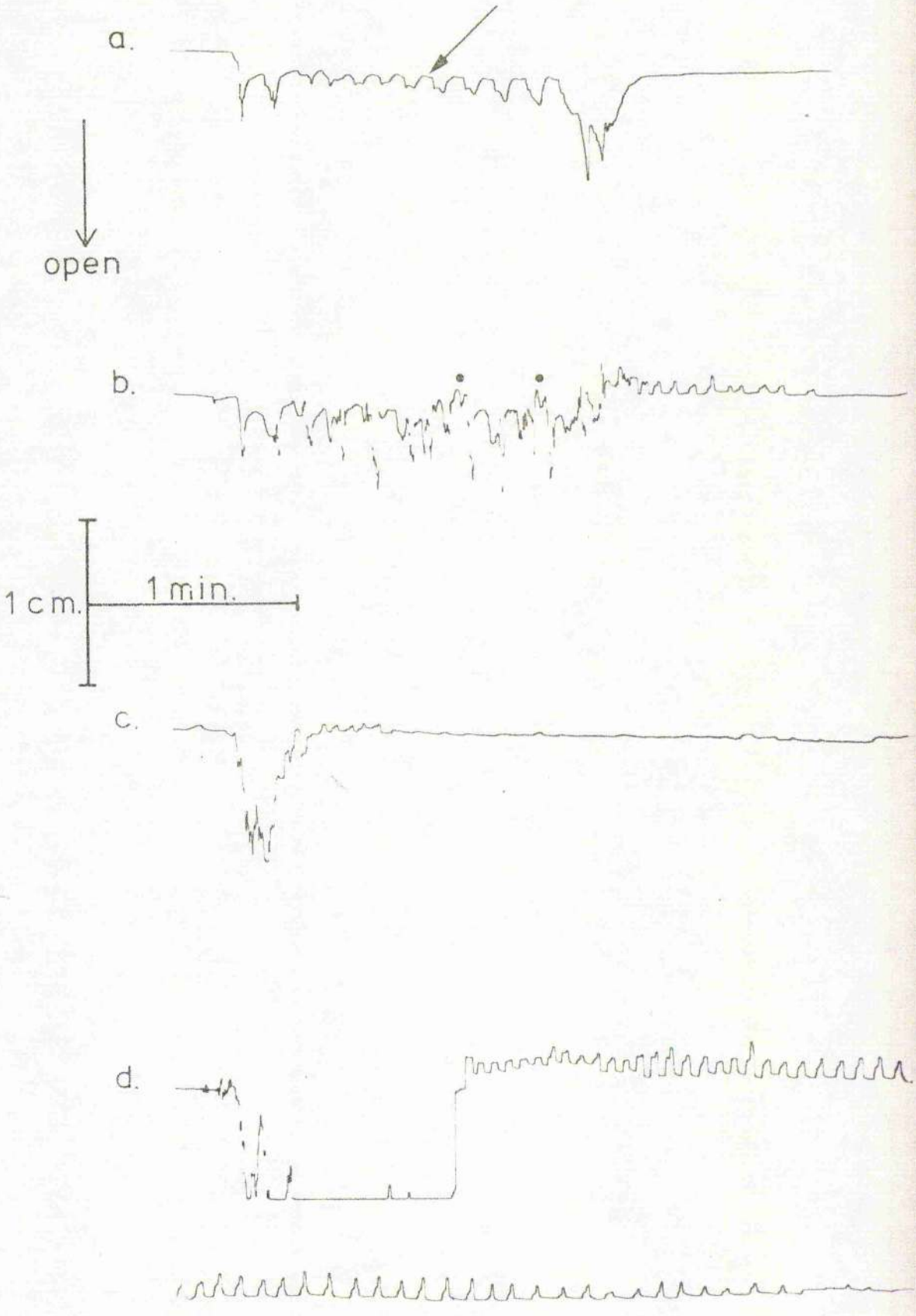

a.
open
b.
1 cm.
1 min.
c.
d.

Figure 39

Swallowing activity. Instantaneous frequency plots of 4 short duration swallowing sequences.

(Details in text)

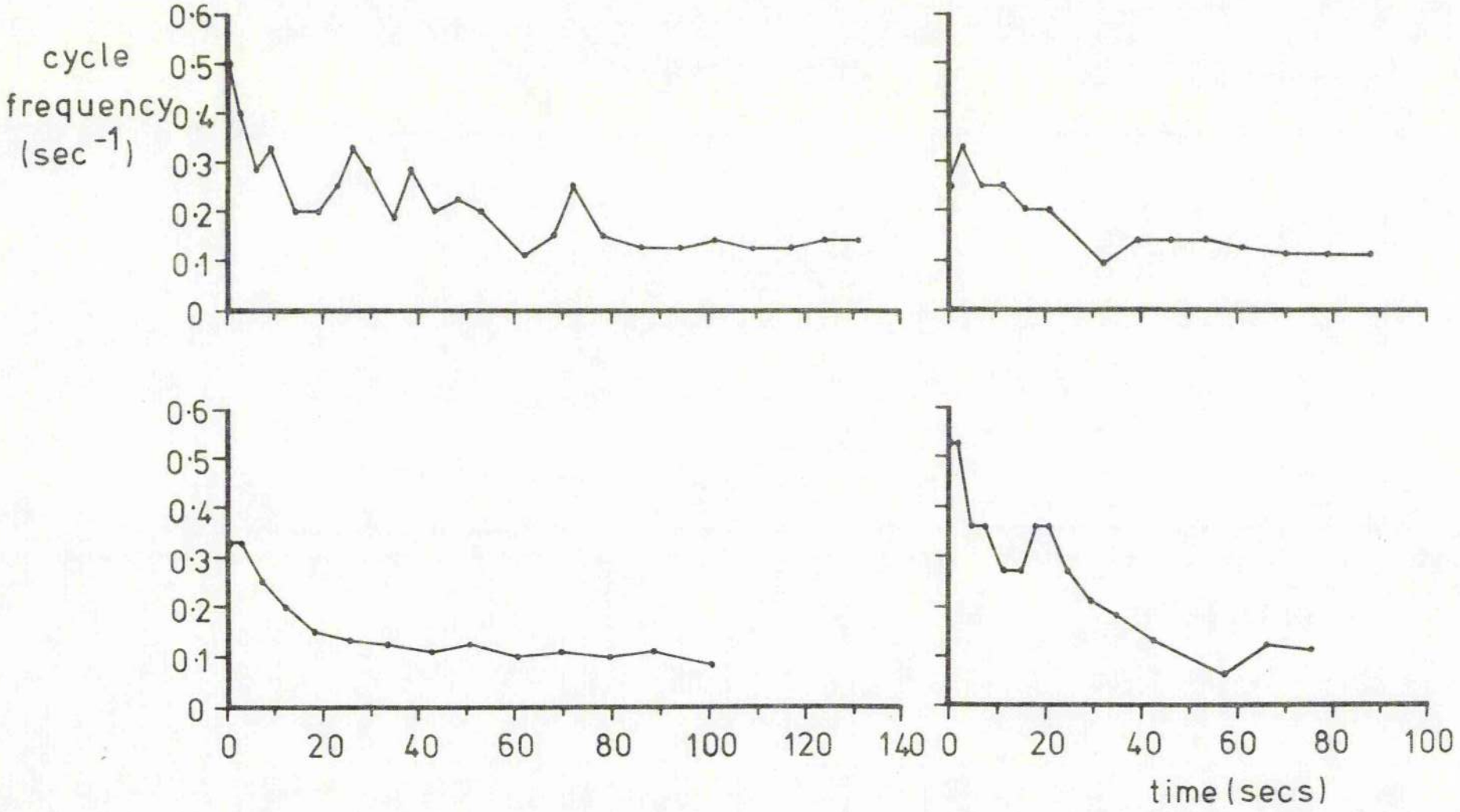

cycle frequency (sec^{-1})
0·6
0·5
0·4
0·3
0·2
0·1
0
0 20 40 60 80 100 120 140
0 20 40 60 80 100
time (secs)

Figure 40

Swallowing activity. Block histogram, for 8 samples, of the number of cycles in 30 sec. bins. Bars indicate standard errors.

(details in text)

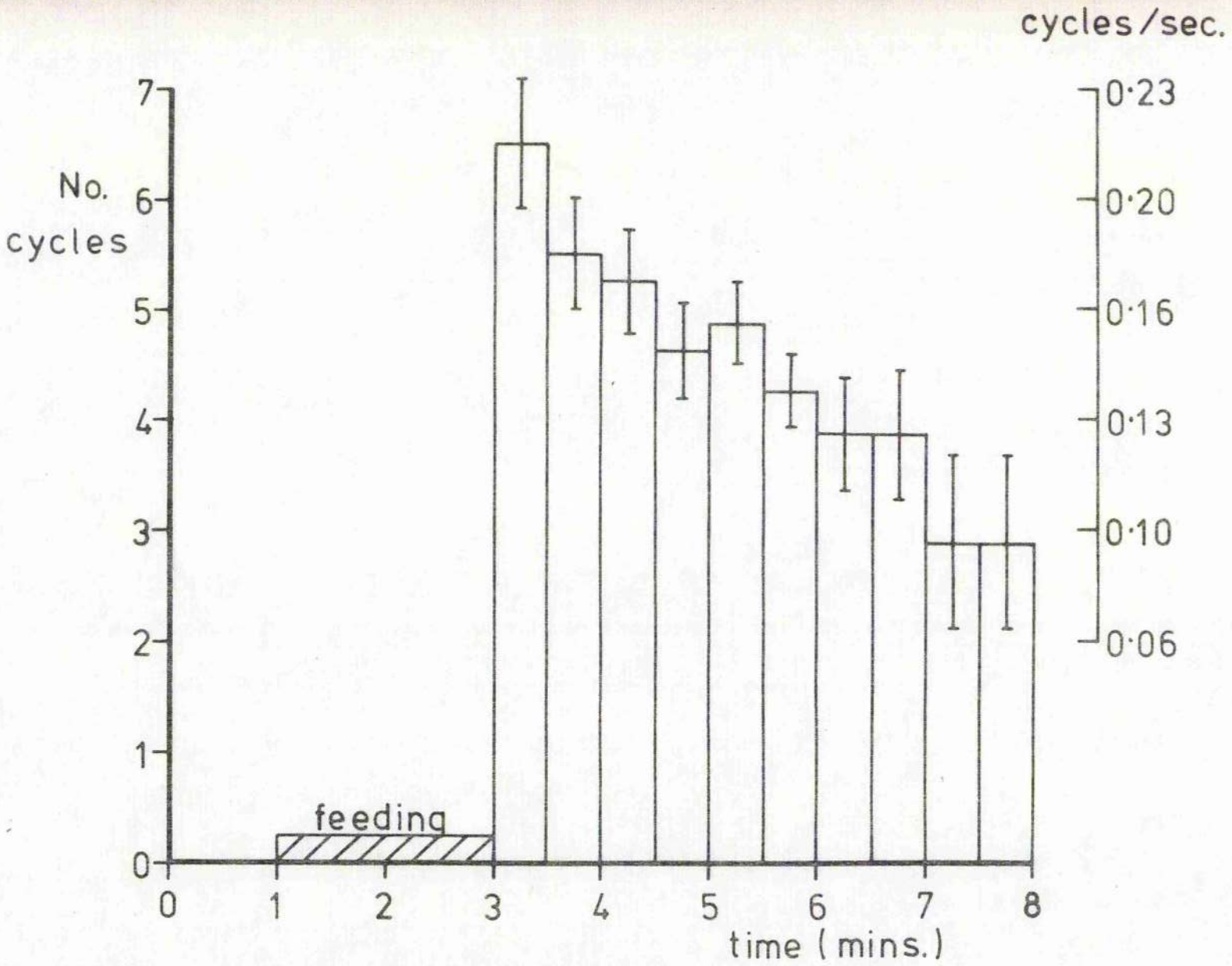

cycles/sec.
No. cycles
7
6
5
4
3
2
1
0
0·23
0·20
0·16
0·13
0·10
0·06
feeding
0
1
2
3
4
5
6
7
8
time (mins.)

Figure 41

Swallowing activity. Kymograph trace of longest recorded sequence. Downward deflection of trace indicates retraction (opening) of the labrum. Ingestion is followed by a long period when small protraction movements of the labrum can be seen.

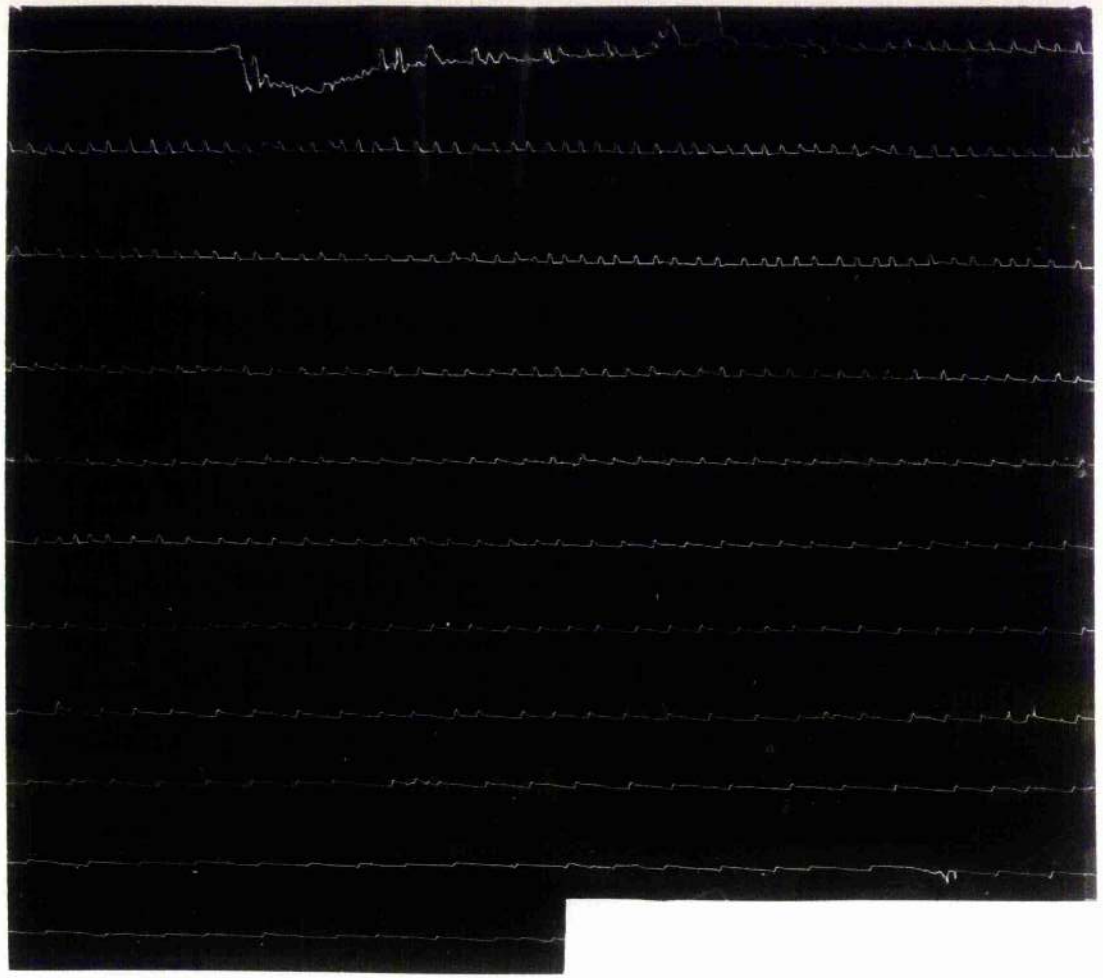
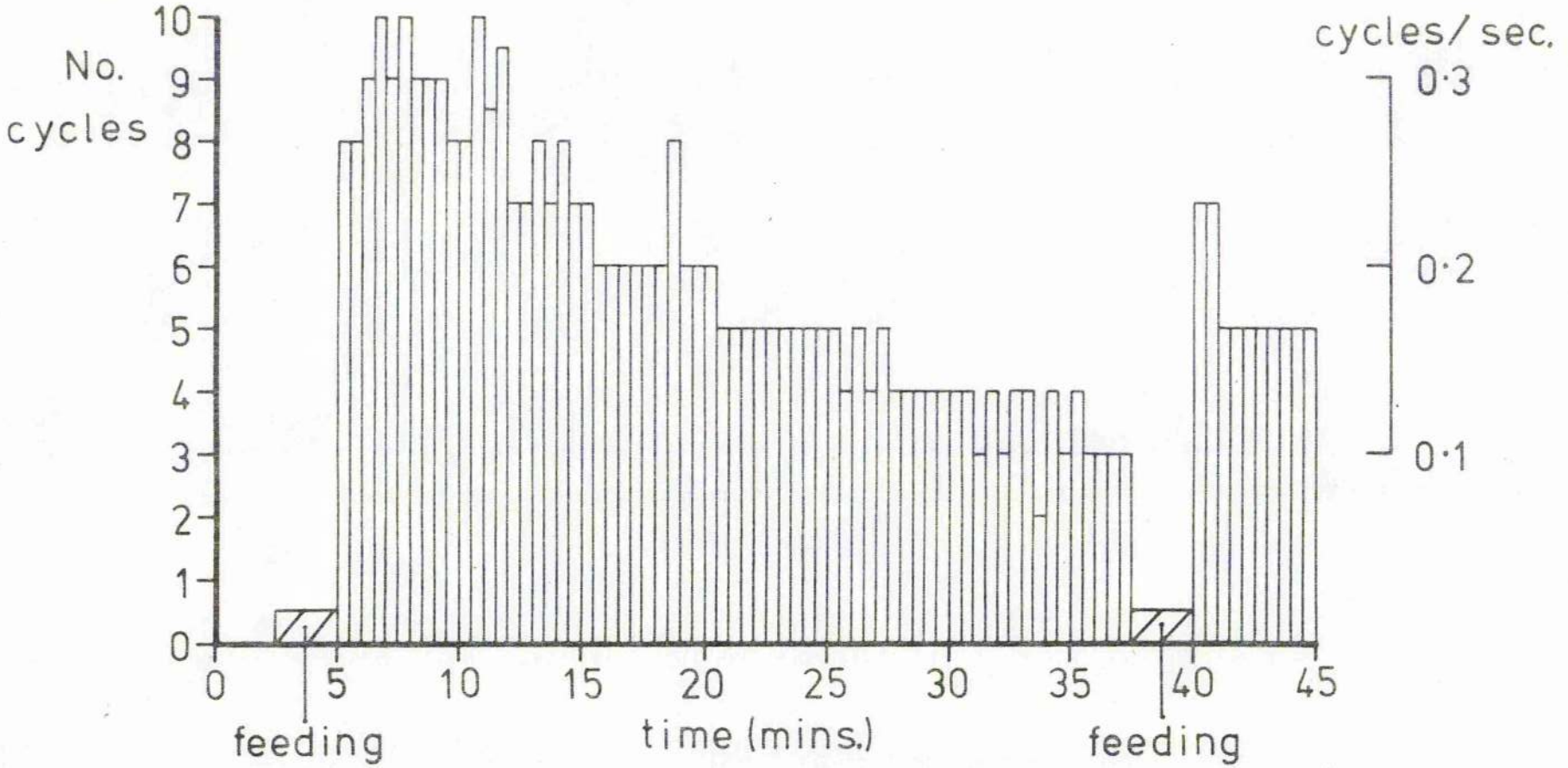

open
1 cm.
1 min.

Figure 42

Swallowing activity. Block histogram of the number of cycles in successive 30 sec. bins for the longest recorded swallowing sequence (Figure 41).

(Details in text)


No. cycles
10
9
8
7
6
5
4
3
2
1
0
cycles/ sec.
0·3
0·2
0·1
0
5
10
15
20
25
30
35
40
45
feeding
time (mins.)
feeding

4. DISCUSSION

Two main problems are encountered when one attempts to interpret the results obtained in this chapter. Firstly, how much of the recorded movements are artefacts passively imposed by the movements of the food in the mouth? This will be particularly important for the 'chewing' phase. Secondly, are the labral movements seen during the 'swallowing' sequence a consequence of oesophageal peristalsis or a true representation of labral activity?

The first problem can be dealt with fairly rapidly by consideration of the trace produced when an elastic substrate takes the place of food (Fig. 38a). A clearer trace is produced because the piece of rubber has a simple shape with no projections, because it does not break and thus because no pieces of the substrate are swallowed. During a bite of the mandibles on the rubber it will be held firmly by the mandibles and the third maxillipeds and its movement will be minimal. Therefore the movements of the labrum during biting are most likely brought about actively and not passively imposed by the substrate. While spurious movements are undoubtedly recorded during the chewing of foodstuffs, the basic pattern of biting and manipulative phases can still be seen.

The second problem is more difficult to resolve. The disposition of muscle O1a and its attachment to the furcular sclerite of the labrum are such that its action will be to retract the labrum as well as dilating the ventral limit of the oesophagus. Furthermore O4a is an intimate bundle of the oesophageal constrictor (O4) and they probably contract simultaneously. The attachment of O4a to the furcular sclerite of the labrum will ensure that labral protraction will occur at the same time as oesophageal constriction. The frequency of the swallowing activity of the labrum (small protractions after closure) is similar to the frequency of the bursting activity which is

recorded in the s.o.n. and correlated with oesophageal peristalsis (see next chapter). It is therefore possible that the small protractions of the labrum are produced by oesophageal movement rather than any activity of the labral musculature. However, each cycle is biphasic which could suggest that at least two muscles are active to produce it. If this is the case, then L6 is the most probable contender for this action of aiding C4. The movements recorded during the chewing sequence are too large to be accounted for merely by oesophageal movements and active labral movements must be incorporated. The problem can only definitely be resolved electrophysiologically, preferably by the implantation of extracellular copper wire electrodes into the muscles of the intact animal. This will be virtually impossible to achieve for most of the labral muscles due to their size and position. Also because the wires will be fouled by the mouth appendages and by the food material and their presence would probably prevent natural feeding. This study has shown that a pin with an attached thread can be implanted in the labrum without interfering with normal feeding, but the only muscle accessible by the route used is L4, the large labral retractor. There would be little value in recording solely from this muscle.

The above attempt to deduce which muscles produce the recorded labral movements is redundant when considering the possible functions of the labrum. That the labrum does move is indisputable, how these movements are effected is to a certain extent irrelevant in this context. Before discussing the role of labral movements further it is necessary to emphasise one point. Labral movements cannot be divorced totally from oesophageal movements. The ventral limit of the oesophagus is the mouth and it is bounded by the labrum, mandibles and paragnaths. Thus, retraction of the labrum to open the mouth will dilate the oesophagus, and dilatation of the oesophagus to open the mouth will retract the labrum. The same is true for labral protraction,

mouth closure and oesophageal constriction. Perhaps therefore, the results reported in this chapter are better described as recordings of the movements of the mouth in the anterior/posterior plane, incorporating movements of the labrum. This concept of the labrum and ventral oesophageal rim serving simply as parts of the mouth has been touched on by Moulins, Dando and Laverack (1970) with reference to the MPR system. They state .... "It can be argued, therefore, that this (the responses of the MPR system) represents a non-specific form of input regarding the positions of the mouth. Changes in the labrum, paragnath, buccal walls and mandible all affect the output from these organs." However they still retain the distinction between the movements of parts of the mouth from that of the mouth itself. Despite the fact that MPR1 and MPR2-3 "constitute different functional groups"; "respond differently to different movements"; "give information via different pathways to different parts of the C.N.S."; "respond asynchronously to the same movement"; and "may also respond in opposing ways": it is possible to go further and consider the MPR system as a single receptor monitoring the size and shape of the mouth, with different cell groupings being primarily concerned with different parts of the mouth. Bearing this interpretation of its function in mind, the naming of the mouthpart receptor system is accurate in that it monitors alterations in position of parts of the mouth. Unfortunately the term mouthpart has come to be associated specifically with the mouth appendages (1st and 2nd maxillae, 1st, 2nd and 3rd maxillipeds) and thus this name may be misleading. The proposed name perioesophageal receptors (Wales, Macmillan and Laverack, 1976a) accurately reflects their position but gives no indication of their function. It is doubtful whether it is necessary to change the name of this system, but if it is then the simple name "mouth receptor" may be appropriate. The concept outlined above undoubtedly has its limitations, but it is useful in the context

of this chapter by eradicating the need to distinguish between movements of the anterior part of the mouth (the labrum) produced by the oesophageal musculature, and those produced by the labral musculature.

The labral movements during chewing are easier to envisage by an analysis of the trace produced on an elastic substrate. The labrum retracts rapidly to accommodate the end of the substrate and then protracts to hold it in place. Thereafter the movements are linked to those of the mandible by protracting during a mandibular bite and retracting between bites, in the manipulative phase. With reference to mandibular activity, Macmillan, Wales and Laverack (1976) have shown that the substitution of food with a similar elastic substrate tends to increase the duration of each biting phase and reduce intervening manipulative phases. They further showed that the mean cycle time (measured from the start of mandibular abduction in successive cycles) increases from 2.19s (s.d. 0.62) on standard substrate (Nerhrops leg) to 3.19s (s.d. 0.70) on elastic substrate, and that there was often a decrease in bite frequency as the sequence progressed. The results reported here for the labrum compare well with these with the exception that in the present work the cycle time on an elastic substrate starts slightly higher at c.4 secs. This difference is of little importance and is probably brought about by different experimental conditions (e.g. the nature of the elastic substrate). Apart from the fact that swallowing activity can be seen to be superimposed on labral chewing activity (indicated by dots in Fig. 38b), only two further differences need be noted. Firstly, between bites on a food substrate the labrum retracts a long way, presumably to allow a new portion of the food access to the mouth: and secondly, the final closing movement with a food substrate is active while that after an elastic substrate has been discarded is probably passive and brought about merely by the elasticity of the structure. To summarise: At each mandibular bite the mouth closes over that part of the

food which is internal to the incisor processes of the mandibles. This may facilitate fracture of the substrate by exerting pressure on it and probably helps to push the fragments of food into the mouth as they are broken off.

It has already been mentioned that the swallowing activity of the labrum has a similar frequency to oesophageal peristalsis. It is believed here that these small protractions exactly mirror each peristaltic wave of constriction of the oesophagus. There is an obvious advantage in these protractions of the labrum. As the mouth closes and a peristaltic wave is initiated the labrum is pulled over the aperture of the mouth, occluding it, and preventing the escape of any food material. It may also help in actively pushing pieces of food into the oesophagus so that the peristaltic wave can have its effect. A discussion of the frequency and duration of this representation of oesophageal peristalsis will have more relevance in the next chapter where these experiments on an intact animal can be compared with those attempting to elucidate the initial and final control of peristalsis.

The results reported here are essentially monitorings of the opening and closing movements of the mouth by recording the movements of the labrum. It has been shown that the labrum co-operates (either actively or passively) in two rhythmical processes (mandibular chewing and oesophageal peristalsis) which occur at different frequencies. Sometimes the labrum is involved in both processes simultaneously. There has recently been a lot of interest in rhythmical motor programmes as useful preparations for analyses of the control of behaviour patterns (for reviews see Moffett, 1977 and Macmillan, 1977). For a consideration of labral activity in these terms it is not really necessary to abandon the idea of the labrum as merely the anterior part of the mouth. This is a functional concept. What one must analyse is the contribution of different muscle groups (and thus possible rhythmic motor output to these groups) to the observed movements, and not the effect of these movements.

If the labral musculature is rhythmically driven during both mandibular chewing and oesophageal peristalsis then the central integration of the two motor programmes would be of considerable interest, especially if the same muscles were active in both. It will be shown in the next chapter that rhythmical bursting activity of the same frequency as that promoting peristalsis can be recorded from the i.l.n. This would tend to suggest that the labral musculature actively co-operates in swallowing activity. One can only surmise about the role of the labral musculature during mandibular chewing. One possibility is that it is driven by a rhythmical motor programme modified by afferent information from the labral receptors and the MPR system. An equally valid proposition is that it is driven solely by simple reflex arcs activated by these receptors. The latter is more attractive as it could easily take account of the great variation in the length of bite cycles with different structures.

Due to the imperfections inherent in the recording system and due to the fact that only movements were monitored, it is dangerous to try and extract too much detailed information from the results. Briefly, this study shows that the labrum takes a part in both mandibular chewing and oesophageal peristalsis and makes some suggestions regarding its role in these activities. Also, it indicates areas where further research could profitably be done.

CHAPTER 4

PHYSIOLOGY

PHYSIOLOGY

1. INTRODUCTION

The foregut of decapod crustaceans comprises a short oesophagus joining the mouth to an ectodermal, chitinised cardiac sac. The cardiac sac contains a complex gastric mill for trituration of food material (see e.g. Balss, 1941). At its posterior limit is a pyloric press and filter which diverts small food particles into the hepatopancreatic ducts and larger food particles into the mid gut (Venk, 1960). The anatomy of the labral/oesophageal complex is presented and reviewed in Chapter 2 of the present work and that of the gastric mill and pylorus has been described in a wide variety of decapod crustaceans by Maynard and Dando (1974), Meiss and Norman (1977a,b) and Pryer (1977). Food is transported peristaltically up the oesophagus and stored in the cardiac sac before it is broken up by the action of the gastric mill. This is claimed to be an adaptation to a sedentary existence, enabling the food to be swallowed first and then chewed at leisure when the animals are safe from predation (Patwardhan; Reddy in Venk, 1960). This chapter is primarily concerned with a small part of the passage of food through the foregut: the control of oesophageal peristalsis. Furthermore, evidence will be presented that contact chemoreceptors play a major part in initiating and terminating oesophageal peristalsis. For these reasons, and to provide a framework from which to work, brief reviews of the foregut neurophysiology and chemosensory mechanisms of decapod crustaceans will appear here.

Foregut Neurophysiology of Decapod Crustaceans

Maynard and co-workers pioneered the work on the stomatogastric system (Maynard, 1966; Maynard and Burke, 1966; Maynard, 1967; Maynard and Atwood, 1969; Morris and Maynard, 1970). In these early studies it was suggested that the stomatogastric system of large decapod crustaceans was an ideal preparation for the analysis of a small neural network which controls a well-defined behavioural

act. Since then a great deal of information about the system has been obtained from a variety of decapod crustaceans. It has been tacitly assumed that the systems in these animals are substantially similar.

1) The stomatogastric ganglion contains 30 neurons and controls the gastric mill rhythm (for triturating food) and the pyloric rhythm (for filtering food) (see e.g. Selverston, 1974 - a review of the system in Panulirus interruptus). These rhythms are produced by alternating bursts of activity in several neurons acting on the striated musculature of the stomach. It has been suggested that local subthreshold presynaptic depolarisations as well as spikes contribute to the normal function of the ganglion in Panulirus argus (Maynard and Walton, 1975). The innervation pattern and neuromuscular physiology of the foregut musculature has been described for Callinectes sapidus and Panulirus argus (Govind, Atwood and Maynard, 1975; Jahromi and Govind, 1976), and evidence has been presented that Acetylcholine acts as the chemical transmitter in at least some stomatogastric neuromuscular junctions of Panulirus interruptus (Marder, 1974).

2) The gastric mill rhythm of Panulirus interruptus is dependent on a network of 12 neurons which can be functionally divided into two subsets: 4 motoneurons driving the lateral teeth, and 6 motoneurons driving the medial tooth. There are two interneurons common to both subsets. The synaptic coupling between these neurons has been elucidated to a large extent (Mulloney and Selverston, 1974a; Selverston and Mulloney, 1974). Reciprocal inhibition among the neurons of the lateral teeth produces a pattern of alternating bursts in the absence of all synaptic input and without the provision of an endogenous burster or any neuron which might act as a master clock. This pattern affects one of the interneurons common to both subsets, thus causing activity in the neurons driving the medial tooth. (Mulloney and Selverston, 1974b). Also it is

suggested that the temporal parameters of accommodation and postinhibitory rebound will determine the duration of the bursts and the repetition rate of the pattern (Mulloney and Selverston, 1974b). Comparative anatomical studies with Penaeus japonicus, Palaemon serratus, Palinurus vulgaris and Homarus americanus show that the 12 neuron gastric mill network is present in shrimps, as well as the larger decapods, although associated with functionally and anatomically different muscles (Sudrie, 1976).

3) The pyloric rhythm of Panulirus interruptus is controlled by a network of 14 neurons, 13 of which are known to be motoneurons. The exception is the anterior burster neuron. The destination of the axon of this neuron is unknown at the present time. This neuron pool is functionally organised into dilators and constrictors which produce alternating bursting output to antagonistic muscles. The basic rhythmicity of the pattern is set by a group of 3 endogenous bursters which can produce cyclic motor output in a totally deafferented preparation. The phase relationships in the other neurons are maintained by their synaptic connectivity. These synaptic interactions are either inhibitory or electrotonic (Maynard and Selverston, 1975).

4) By concentrating on the motor output of the stomatogastric ganglion, Powers (1973) (with Cancer magister and C. productus) and Hartline and Maynard (1975) (with Panulirus argus) have investigated the functional implications of the generated patterns.

5) Norris and Maynard (1970) and Powers (1973) using electrode implantation techniques studied the stomatogastric output in intact Homarus americanus and Cancer spp. respectively. They showed that although the recorded discharge is similar to that described from isolated preparations, the rhythmic patterns are also under the control of modulating interneurons from the C.N.S.

and probably pathways of reflex sensory feedback. Larimer and Kennedy (1966) described an unusual mechanosensory bipolar cell which is located in the stomatogastric ganglion. Its autogenic activity is modulated by movements of the gastric mill ossicles. These movements are also monitored by groups of sense cells located in the posterior stomach nerve (p.s.n.)(Dando and Laverack, 1969). The effect of p.s.n. stimulation on normal gastric and pyloric rhythms in Cancer pagurus (Chanussot and Dando, 1973) has been described (Dando, Chanussot and Nagy, 1974). That these effects are compatible with the theory that p.s.n. stimulation reflexly activates command fibres which act only on the pyloric dilator pacemaker neurons has been confirmed by Hermann and Dando (1977). They propose that the modification in the pyloric output may be associated with an opening of the cardio-pyloric valve and a rapid propulsion of food through the pyloric filter into the midgut. The command fibres derive from the commissural ganglia and may be similar to the commissural ganglion E neurons described by Russell (1976) in Panulirus interruptus. These, however, act on the medial tooth neuronal subset. Russell considered that the force exerted by the medial tooth of the gastric mill could be adjusted by central and sensory modulation of the E neurons. Modulation of the stomatogastric ganglion output of Panulirus argus, can be effected by stimulation of the two inferior ventricular nerve through-fibres (command fibres from the cerebral ganglia), although the function of these changes is unknown (Dando and Selverston, 1972).

6) Movements of the cardiac sac of Palinurus vulgaris are controlled by three motoneurons. One (AM) innervates the intrinsic musculature and the other two (CD1 and CD2) innervate the extrinsic dilators. The cell body of CD1 is contained in the oesophageal ganglion whereas that of CD2 can be found in the stomatogastric ganglion. Moreover, CD2 has two spike initiation sites, one in each of the stomatogastric and oesophageal ganglia. It is proposed that it "a) might be involved in various motor programmes" and; "b) could be considered

as a 'two-way co-ordinating system'." (Vedel and Moulins, 1977).

7) Knowledge of the neuronal mechanisms co-ordinating oesophageal peristalsis is limited. It has been documented that the oesophageal musculature is innervated by rhythmical bursting activity (Moulins, Vedel and Dando, 1974 for Palinurus vulgaris; Spirito, 1975 for Procambarus clarkii; Russell (unpublished) in Selverston, Russell, Miller and King, 1976 for Panulirus interruptus). In Palinurus vulgaris the oesophageal musculature is innervated by 3 dilator (O.D. 1,2 & 3) and 2 constrictor (C1 & 2) motoneurons. The cell body of at least one of the dilator motoneurons (O.D.1) is located in the oesophageal ganglion. The output of this oesophageal network can be modified by activity of the cardiac sac network, especially by activity of C.D.2, which is considered as a command interneuron as well as the pacemaker of the cardiac sac network (Moulins and Vedel, 1977).

8) Finally, Russell ((unpublished) in Selverston, Russell, Miller and King, 1976) has shown that stimulation of an oesophageal chemoreceptor nerve in Panulirus interruptus can modulate both the gastric and oesophageal rhythms. This takes the form of a 2-3 fold increase in cycling and suggests that the two rhythms may be coupled in some instances. It is worth mentioning here that this chemoreceptor of Panulirus interruptus is possibly homologous to the A.O.S. of Homarus gammarus. Evidence will be presented in this chapter that stimulation of the a.o.s.n. of Homarus gammarus decreases the frequency of the oesophageal rhythm. Reasons for this will appear later.

The above review is not particularly detailed. Selverston, Russell, Miller and King (1976) have provided an extensive review of the stomatogastric nervous system (with emphasis on Panulirus interruptus) and Wales (1976) has reviewed arthropod gut proprioceptors which could provide modulatory inputs to the system.

Chemoreception in Decapod Crustaceans

The profusion of recent reviews which have relevance for decapod crustacean chemoreception (Laverack, 1968; Lindstedt, 1971; Laverack, 1974a,b; Mackie, 1975; Laverack, 1975; Ache, 1977) is such that a detailed consideration of the subject here is unnecessary. This section serves simply to summarise some of the available information.

Laverack (1974b) defines chemoreceptors as "... receptor cells specialized for the detection of small changes in the chemical composition of the environment due to substances volatile, soluble or electrolyte. Such cells are normally constituents of the nervous system, or are directly associated with the secondary neurones that act as conduction pathways to the C.N.S." This definition will be used here. Two types of chemoreceptor are proposed: low threshold (olfactory) and high threshold (gustatory) (Laverack, 1975). The former are typified by the antennular aesthetasc hairs (Laverack and Ardill, 1965, for Panulirus argus; Ghiradella, Case and Cronshaw, 1968a, for Coenobita compressus; Ghiradella, Cronshaw and Case, 1968, for Pagurus hirsutiusculus) and the latter by chemoreceptors on the dactyls and mouthparts (Shelton and Laverack, 1968, Shelton and Laverack, 1970 for Homarus gammarus). The structure of these organs is essentially similar but that of the OS (Chapter 2, this dissertation) is markedly different, resembling to a greater extent the contact (gustatory) chemoreceptors described in insects (Moulins, 1968; Moulins, 1971; Cook, 1972).

To date only one type of chemoreceptor has been extensively studied. This is the antennular aesthetasc hair organ. Each filamentous hair is innervated by 300-400 dendrites and the stimulatory materials probably gain access to the dendrites through the permeable hair wall (Ghiradella, Case and Cronshaw, 1968b; Laverack, 1975). Shelton, Shelton and Edwards (1975) from studies on the infection of the aesthetasc setae of Crangon crangon by a

filamentous, epizoic bacterium, present evidence that, in these animals, aesthetasc setae do not have a terminal pore. The antennules have been shown electrophysiologically to be sensitive to the lower molecular weight components (amines and amino acids) of food material (e.g. Levandowsky and Hodgson, 1965). Behavioural responses, which are mediated in part by the antennular chemoreceptors, also show that amines and amino acids are effective stimulants, especially in mixtures (McLeese, 1970; Mackie and Shelton, 1972; McLeese, 1973). Carr and Curin (1975) have shown that larger molecular weight components such as proteins are also potent stimulants of feeding behaviour in Palaemonetes pugio. The eyestalk ganglia have several non-visual functions (Hazlett, 1971), and one of them, the medulla terminalis, is involved in processing the chemosensory input from the antennules (Maynard and Dingle, 1963, Maynard and Yager, 1968). Bilateral ablation of the medulla terminalis in Panulirus argus causes: an increase in the positive response to dactyl stimulation; an increased tendency for the animal to mouth and ingest inedibles; and a disturbance of normal ingestion (Maynard and Wallace, 1970).

In spite of the fact that chemosensitivity is probably involved in a number of diverse activities (e.g. feeding, reproduction and homing), knowledge of the mechanisms whereby this sensitivity controls the behaviour of decapod crustaceans sadly is lacking. Behavioural experiments can provide one with a long list of stimulatory compounds, and simple recording experiments can characterise the responses of individual receptor cells to these compounds (Shepheard, 1974). It is necessary now to investigate how these responses are integrated with normal neuronal activity to produce the modulation of behaviour. Laverack (1975) pleads for more attention to be paid to crustacea in the search for fundamental principles of chemoreception.

Objects of Research

1. Three small bilateral groups of bipolar sense cells have been described innervating the labrum (Chapter 2). The first object of this electrophysiological study was to confirm that they respond to mechanical deformation of the labrum.

2. The review of foregut neurophysiology of decapod crustaceans shows that information about the control of oesophageal peristalsis is lacking. An attempt was made to characterise the bursting activity recorded from the oesophageal nerves during peristalsis.

3. The studies on the labral movements during feeding (Chapter 3) indicated that the labrum undergoes small rhythmic protractions at a frequency similar to that of oesophageal peristalsis. It was uncertain whether or not this movement was due to an active participation of the labral musculature. Recordings were taken from the nerves innervating the labrum to determine if the labral musculature is driven by rhythmical neuronal activity during oesophageal peristalsis.

4. Two bilateral chemoreceptor organs have been described at the oesophageal/cardiac sac valve (Chapter 2). Their position suggests that they may be involved in the control of ingestion. Furthermore, labral swallowing activity (oesophageal peristalsis) is initiated only after some food material has been bitten off the substrate and pushed into the oesophagus: not during mandibular chewing, and not by chemical stimulation of the mouthparts (Chapter 3). This implies that there is an internal mechanism for the initiation of peristalsis. The main concern of this chapter is an investigation of the feedback effects of P.O.S. and A.O.S. stimulation. This has relevance in the study of both the control of oesophageal peristalsis and the role of chemosensory afference in behaviour.

2. MATERIALS AND METHODS

Preliminary Dissection

The chelae, abdomen, pereiopods and 3rd maxillipeds were removed from a healthy animal (there was no discrimination on the bases of size or sex). Two lateral cuts, one on either side of the body, and one transverse cut, just posterior to the rostrum, were made, and the carapace was removed by scraping it free of the various muscle insertions. The major mandibular adductor muscles (M9 of Walen, Macmillan and Laverack, 1976a) on both sides were removed by cutting their apodemes. Also removed at this stage were the digestive glands and gonads and the dissection was sluiced with fresh sea water to remove any secretions which may have escaped from them. To free the stomach from the rostrum, the origins of the cardiac stomach and gastric mill muscles arising from the precephalic apophysis (Hocquard, 1883) and the eye cups, (gm1b; C1 and C2 of Maynard and Dando, 1974) were scraped free of the cuticle using a sharp mounted needle. The stomach could then be reflected posteriorly allowing access to the green glands at the base of each antenna. The green glands were removed, taking particular care to ensure that no part of them remained, and the dissection was washed with fresh sea water. The thorax was divided transversely by cutting just posterior to the cephalic apodeme of the endophragmal skeleton and the metastomal plate of the ventral skeleton. The anterior part was pinned through the antennae into a wax bottomed dissection dish containing fresh sea water. Then the circumoesophageal connectives on either side of each commissural ganglion were cut to isolate the stomatogastric, and oesophageal nervous systems from higher control. Further dissection was performed under sea water with the aid of a Nikon binocular dissection microscope.

Dissection to Reveal the Oesophageal Nervous System

Having reached the position described above it was relatively simple to reveal the oesophageal nervous system of one side. Muscles C4, C5, CV1 and OCSV3 were cut near their insertions on the oesophagus. This enabled the stomach to be deflected postero-laterally and fixed in this position by pinning it through the pylorus. The procedure was then to dissect further as little as possible for each particular experiment. To gain access to the:

(a) s.o.n. - no further dissection was necessary, although cutting O3 helped;

(b) v.-p.o.n. - O5 was cut;

(c) s.o.s.n. - O5, OCSV1 and OCSV2 were cut;

(d) i.o.n. and the i.l.n. - O3, O5, OCSV1, OCSV2 and O2 were cut, and the oesophagus deflected posteriorly.

Responses of the Labral Receptors - Dissection and Stimulation

The animal was dissected to stage (d) above. Then the cephalic apodeme was removed by cutting it in the longitudinal midline and excising each half. To remove the mandible from one side the outer mandibular nerve was cut at the point where it leaves the circumoesophageal connective; the lateral wall of the oesophagus was cut free from the groove on the inner rim of the mandible; and the epistoma was cut transversely from its lateral edge to the point where the mandible hinges with it. The preparation was then tilted slightly and a small incision was made in the lateral rim of the oesophagus to reveal the lateral wall of the labrum. In this position it was possible to record from the i.l.n. of one side and stimulate mechanically the ipsilateral side-wall and lobe of the labrum.

An attempt was made to deliver repeatable quantifiable mechanical stimuli to the labrum via a Servomex waveform generator driving a pen arm. However, this proved unreliable as the dissection had destroyed the labral

support on one side, rendering it slightly plastic to imposed movements. All the responses shown in the results were obtained by stimulation with a hand-held seeker.

Recording and Stimulating Techniques

Electrical activity in the nerves was picked up with conventional silver wire hook electrodes, amplified differentially using a type RP/1 pre-amplifier (manufactured at the Catty Marine Laboratory) with high and low frequency cut-offs at 1KHz and 80Hz, respectively and displayed on a Tektronix type 561A oscilloscope. Permanent records were made using a Cossor oscillegraph camera. Selected nerves were stimulated with a Tektronix pulse generator (type 161), which was powered by a Tektronix waveform generator (type 162) and a Tektronix power supply (Type 106A). To reduce stimulus artefact to a workable level, an R/P isolation unit with a full scale deflection of 20 volts was interposed between the pulse generator and the stimulating electrodes (silver-wire hook electrodes). In all stimulating experiments the stimulus was a train of rectangular pulses of width 0.5 - 0.7 ms and with a pulse interval of 400-500ms. In the majority of experiments the bathing medium was sea water, and the nerves were insulated with either liquid paraffin, or a glutinous mixture of liquid paraffin and petroleum jelly. The latter has the advantage of adhering to the nerve and electrodes thus ensuring that sufficient sea water can be returned to the bath to cover the preparation completely. When attempts were made to record chemoreceptor activity from the various sense organs the preparations were bathed in Homarus saline (Pantin, 1964), and glass-tipped suction electrodes were used.

Chemical stimulation of the organs was performed with an extract of Mytilus edulis. The gills and mantle of a fresh mussel were homogenised in approximately the same volume of sea water. This was applied to the appropriate area with a glass pipette. For the P.C.N. and the A.C.N. a hole was cut in the

cardiac sac and the pipette inserted and positioned close to the relevant organ. When the P.C.S. was stimulated chemically the afferent axons of both A.C.S. were cut, and vice versa. For the labrum the whole cardiac sac was removed at its junction with the oesophagus and the pipette was inserted through the O.C.S.V. to deliver a stream of the extract down the oesophagus and over the labral lobe. An artefact associated with the release of the extract provided a reliable indicator of the time of application.

3. RESULTS

Responses of Labral Receptors

Figure 43 shows recordings from the i.l.n. (cut centrally) during various types of mechanical stimulation of the labrum. An attempt was made to stimulate selectively the areas innervated by the 3 groups of sense cells described previously (lobe, side and floor).

Prodding the lobe and side (Fig. 43a and c respectively) of the labrum elicited sharp bursts of receptor activity which terminated rapidly. Both of these populations responded to a stroke along the side from anterior to posterior, ending at the lobe (Fig. 43b). Large sensory units associated with lobar stimulation (Fig. 43a) can be seen at the end of a stroke (Fig. 43b).

Stroking and prodding the scutiform sclerite yielded no response. To stimulate the sensory group associated with the floor, the labrum was extended and flexed (Fig. 43d and e respectively) about the transverse midline. Extension appeared to be the more potent stimulus, eliciting a train of activity whose frequency slowly decayed (Fig. 43d). Flexion provoked a slight increase in the background spontaneous activity. The large units seen in Fig. 43e arose as a result of movements of the probe stimulating the lobar receptors and the large deflection of the trace at the end of the stimulus is an artefact associated with rapid release of the labrum.

Chemical stimulation of the labrum with Mytilus extract produced no response.

Figure 43

Responses of the labral receptors to mechanical deformation of the labrum.

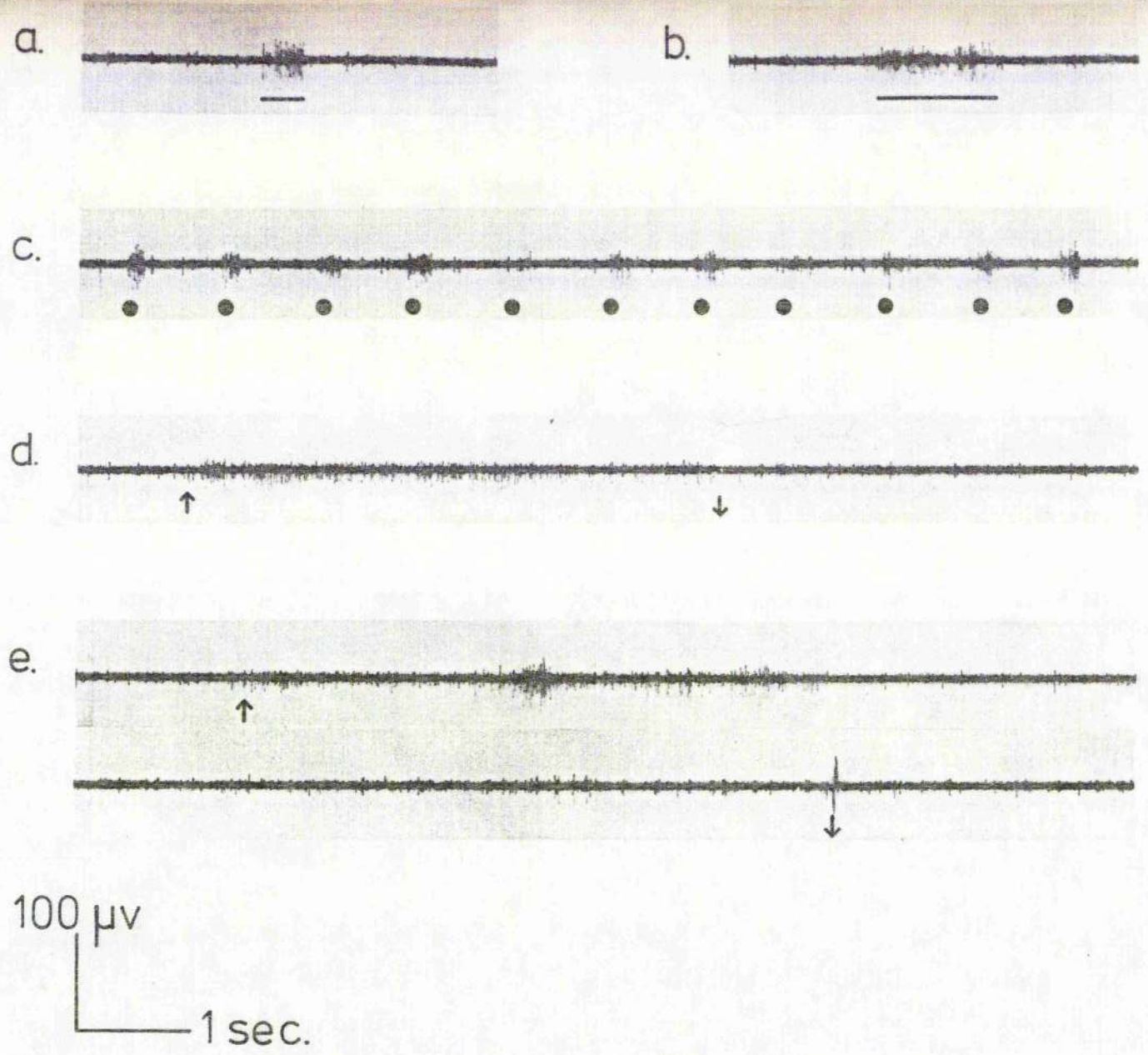
a. Prod to the lobe. Bar indicates duration of stimulus

b. Stroke along the side from anterior to posterior. Bar indicates duration of stimulus

c. Prodding the side in the region of the furcular sclerite. Each prod is indicated by a dot.

d. Extension of the labrum by pulling ventrally on the scutiform sclerite. Arrows indicate initiation and cessation of stimulus

e. Flexion of the labrum by pushing anteriorly on the tip of the scutiform sclerite. Arrows indicate initiation and cessation of stimulus. Bursts of activity during stimulation are probably a result of lobar stimulation with the probe and not a result of labral flexion.


a.
b.
c.
d.
e.
100 µv
1 sec.

Rhythmical Bursting Activity Associated with Peristalsis

The s.o.n., v.-p.o.n. and i.o.n. are the three major nerve trunks originating from the commissural ganglia and innervating the oesophageal and labral musculature. Typical bursting activity recorded from these nerves can be seen in Figs. 44 and 45.

Recordings from the s.o.n. reveal rhythmical bursting activity of at least three neurons during peristalsis. The most conspicuous and easiest to record of these is a large unit which, using an audio link, could be heard to be active during oesophageal dilatation. In all subsequent experiments the burst of this large unit is considered to indicate oesophageal dilatation during peristalsis and is used as a reference point. Also present in the s.o.n. cycle are a medium sized unit which is active during dilatation (closed circles in Fig. 44b) and a small unit which is active between dilator bursts and is presumably a constrictor unit (open circles in Fig. 44b).

The v.-p.o.n. cycle is composed of a small unit which fires in a 1:1 relationship with the small constrictor unit in the s.o.n. (open circles in Fig. 44b), with the s.o.n. unit occurring a few milliseconds earlier. This is terminated by a short high frequency burst incorporating at least two units and occurring about $\frac{1}{2}$ sec. before initiation of the medium s.o.n. unit mentioned above. Also noticeable is a very small unit which corresponds 1:1 with the large s.o.n. unit (open triangles in Fig. 44b). Here also the s.o.n. unit occurs fractionally earlier. In Fig. 50 it can be seen that the structure of the v.-p.o.n. cycle closely approximates that of the s.o.n. cycle in Fig. 44.

While the burst patterns in the s.o.n. and in the v.-p.o.n. are similar, that recorded from the i.o.n. is completely different. Three different units can be distinguished: a medium sized unit which fires in a low frequency burst during the dilator burst in the s.o.n. (open circles in Fig. 45a); a small unit (open triangle in Fig. 45a); and a large unit which can be described as

firing all the time but being inhibited by the medium unit and exhibiting a rebound excitation (closed circles in Fig. 45a). This activity was recorded in the i.c.n. proximal to the i.l.n. branch. Recording on the other side of the i.l.n. branch (between i.l.n. and the oesophageal ganglion) revealed a unit very similar to the s.c.n./v.-p.c.n. constrictor in its size, frequency, and position relative to the s.c.n. dilator burst (Fig. 45b). That this originates from the commissural ganglion and not the oesophageal ganglion can be seen by cutting the i.c.n. as it leaves the commissural ganglion in which case the unit is no longer seen (Fig. 45c). One would presume that most of the rhythmical bursting activity seen in the i.c.n. travels down the i.l.n. to innervate O1 and the labral musculature.

The frequencies of all these described rhythmical bursts are the same at any one time and this varies within the limits, 1 burst every 10 secs (.1Hz) and 1 burst every 3 secs (0.33Hz). An increase in burst frequency is accompanied by a shortening of each burst length and a concomitant increase in the spike frequency within each burst, (see v.-p.c.n. Fig. 50).

Analysis of the traces is limited by the problems inherent in recording from large nerve trunks with simple hook electrodes. For example, the relative sizes of different units depend on which portion of the nerve is in closest contact with the electrodes. The situation is exacerbated by the fact that the preparations were not deafferented and some sensory activity is bound to be present in the traces. As a consequence the above results are used to show only that:

(a) the s.c.n. possesses a recognisable rhythmically bursting unit which corresponds to oesophageal dilation during peristalsis;

(b) there is a variety of rhythmically bursting units in the three major nerve trunks leaving the commissural ganglion and these probably act to promote effective peristalsis. The relationships between individual units within each cycle and the muscles they innervate have not been studied;

Figure 44

Rhythmical bursting associated with peristalsis. Recorded in the s.c.n. and in the v.-pcn.

a and b at different film speeds

open circles - the small constrictor unit in the spn. which fires on a 1:1 basis with the v.-pcn. constrictor unit

closed circles - medium sized dilator unit

open triangles - very small unit in the v.-p.c.n. corresponding 1:1 with the conspicuous large dilator unit in the s.c.n.

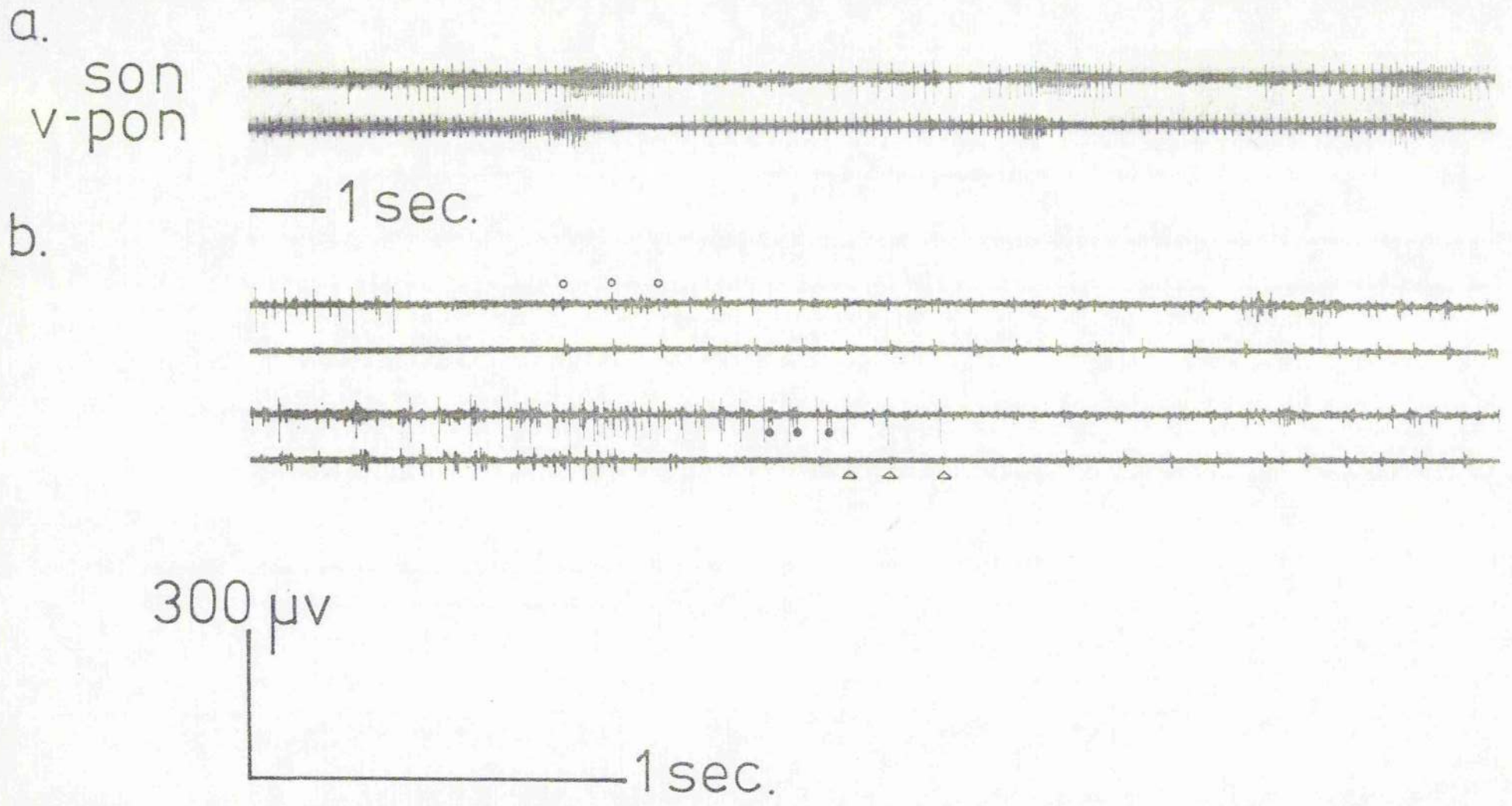

a.
son
v-pon
1 sec.
b.
300 µv
1 sec.

Figure 45

Rhythmical bursting associated with peristalsis. Recorded in the i.c.n. and the s.c.n.

a. recording i.c.n. proximal to i.l.n. to show three units

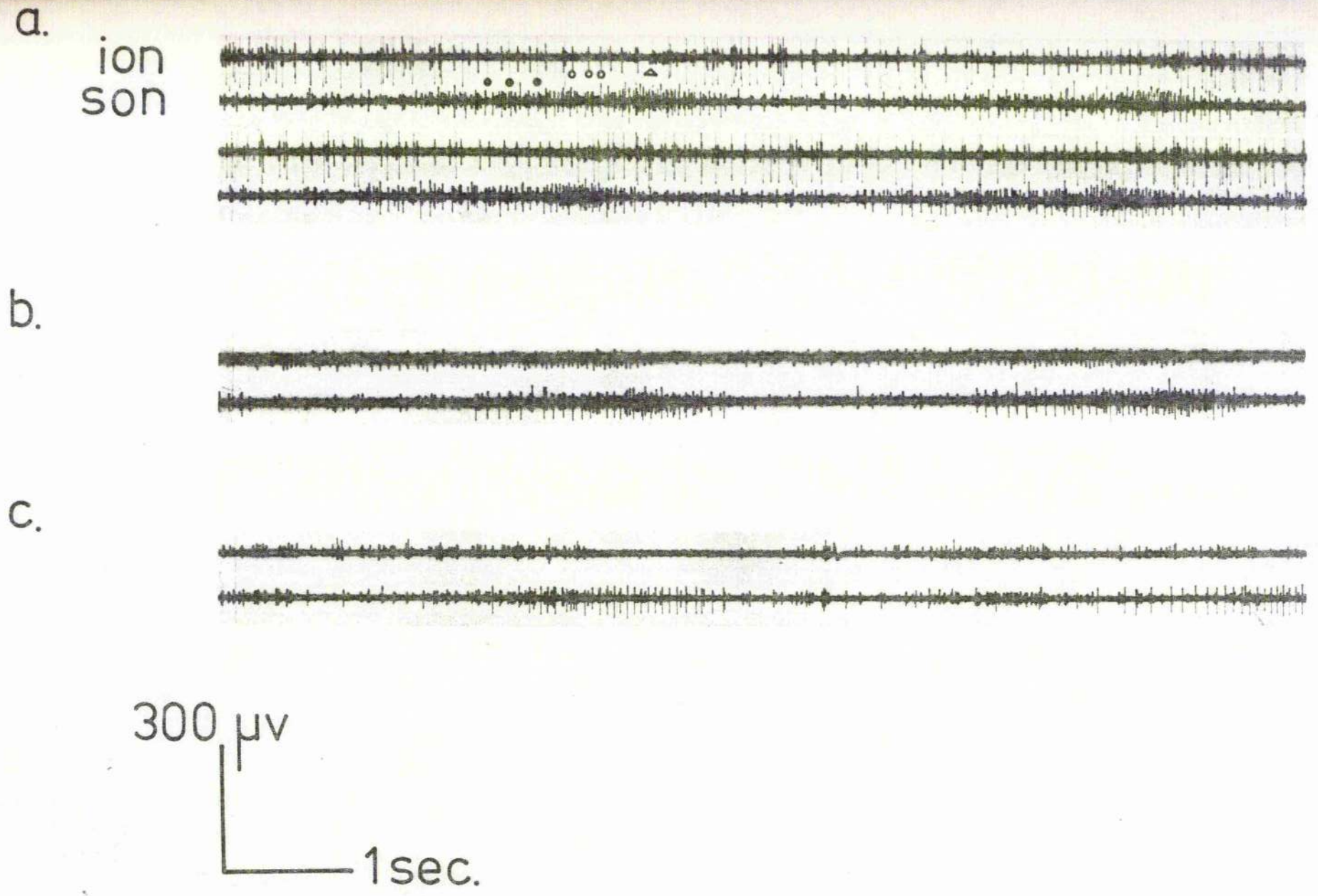
closed circles - large unit firing in a long burst

open circles - medium unit occurring as a low frequency burst during the dilator burst in the s.c.n.

open triangle - indicates the beginning of a short high frequency burst of a small unit

b. recording i.c.n. distal to i.l.n. (between i.l.n. and the oesophageal ganglion)

c. as b but with the i.c.n. cut as it leaves the commissural ganglion


a.
ion
son
b.
c.
300 μv
1sec.

(c) C1 and the labral musculature are innervated by a rhythmically bursting input with a frequency variation between .1Hz and .33Hz during peristalsis.

Feedback Effects of the O.S.

Using the s.o.n. dilator burst as an indicator of peristaltic frequency, the effect of electrical stimulation of the nerves carrying the sensory axons (v.-p.o.n. and a.o.s.n.) and of chemical stimulation to the organs themselves (P.O.S. and A.O.S.) was studied.

(a) Posterior Oesophageal Sensor

Electrical stimulation of the v.-p.o.n. could initiate bursting activity in the s.o.n. (Figs. 46 and 47). This experiment was performed on an ageing preparation which had ceased spontaneous bursting. Three consecutive trains of pulses were delivered to the v.-p.o.n. (Fig. 47A & B). The first caused an increase in the background activity during stimulation and bursting was initiated after cessation of stimulation. The second initiated bursting immediately after the onset of stimulation and this continued after stimulation ceased. Finally, the third train initiated bursting immediately but this ceased during stimulation. The burst frequency in each case was level and low at c.0.15Hz. That the effect was short-lived was probably due to the age of the preparation.

Stimulation of the v.-p.o.n. during spontaneous bursting activity of the s.o.n. caused an increase in s.o.n. burst frequency (Figs. 48 and 49). This was variable during stimulation but after stimulation it settled down to a long-lived stable high frequency at c.0.3Hz. The extent of the effect was dependent on the original level of activity.

No sensory activity could be recorded from the sensory axons of the P.O.S. in the v.-p.o.n. but the effect of electrical stimulation could be mimicked by an application of Mytilus extract directly onto the organ (Figs.50, 51)

Figure 46

Initiation of bursting activity in the s.o.n. on stimulation of the v.-p.o.n. Dots indicate the start of each burst and arrows mark the onset and termination of stimulation.

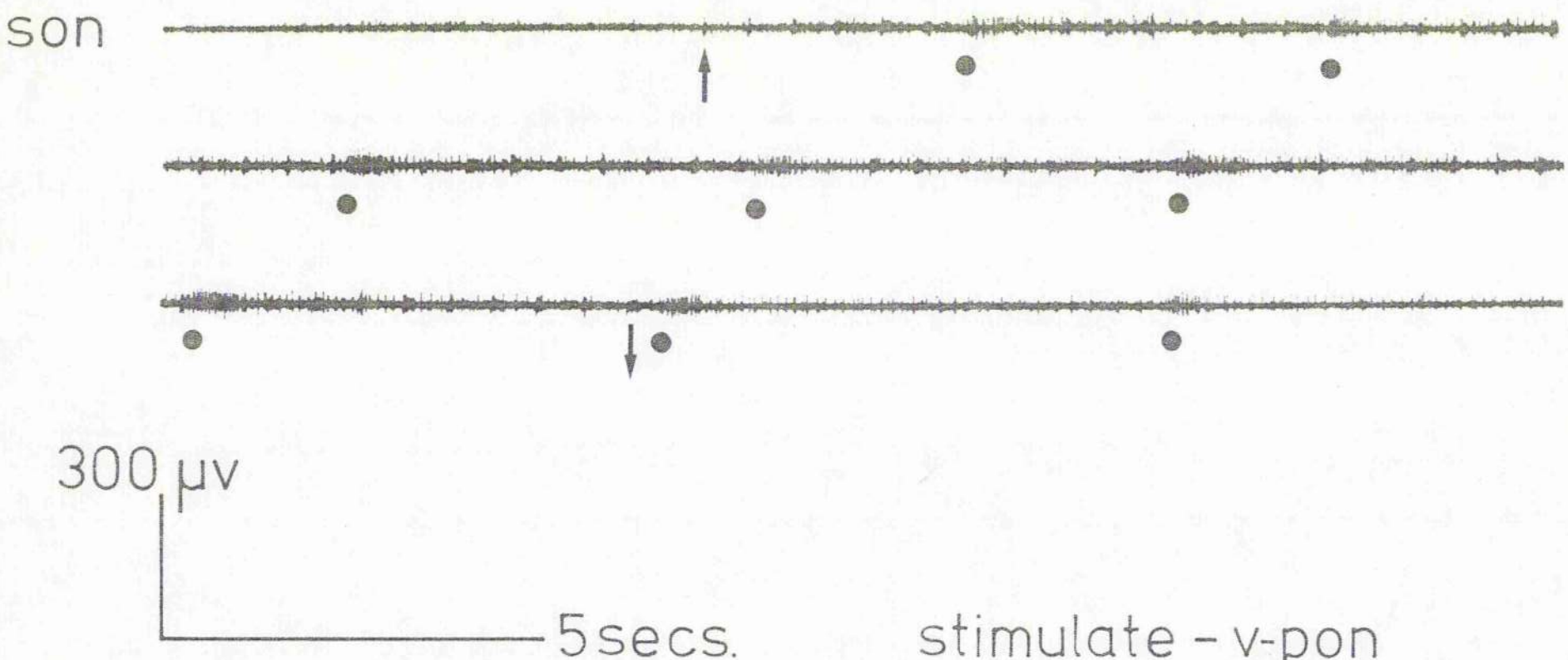

son
300 µv
5 secs.
stimulate - v-pon

Figure 47

Graphs of the s.o.n. burst frequency and a histogram of the number of spikes in successive 1 sec bins during stimulation of the v.-p.o.n. Stippled bars mark the duration of each stimulus. A + B are continuous.

In this and all following graphs, the instantaneous frequency of a burst is calculated as the reciprocal of the interburst interval which is measured from the start of one burst to the start of the succeeding one.

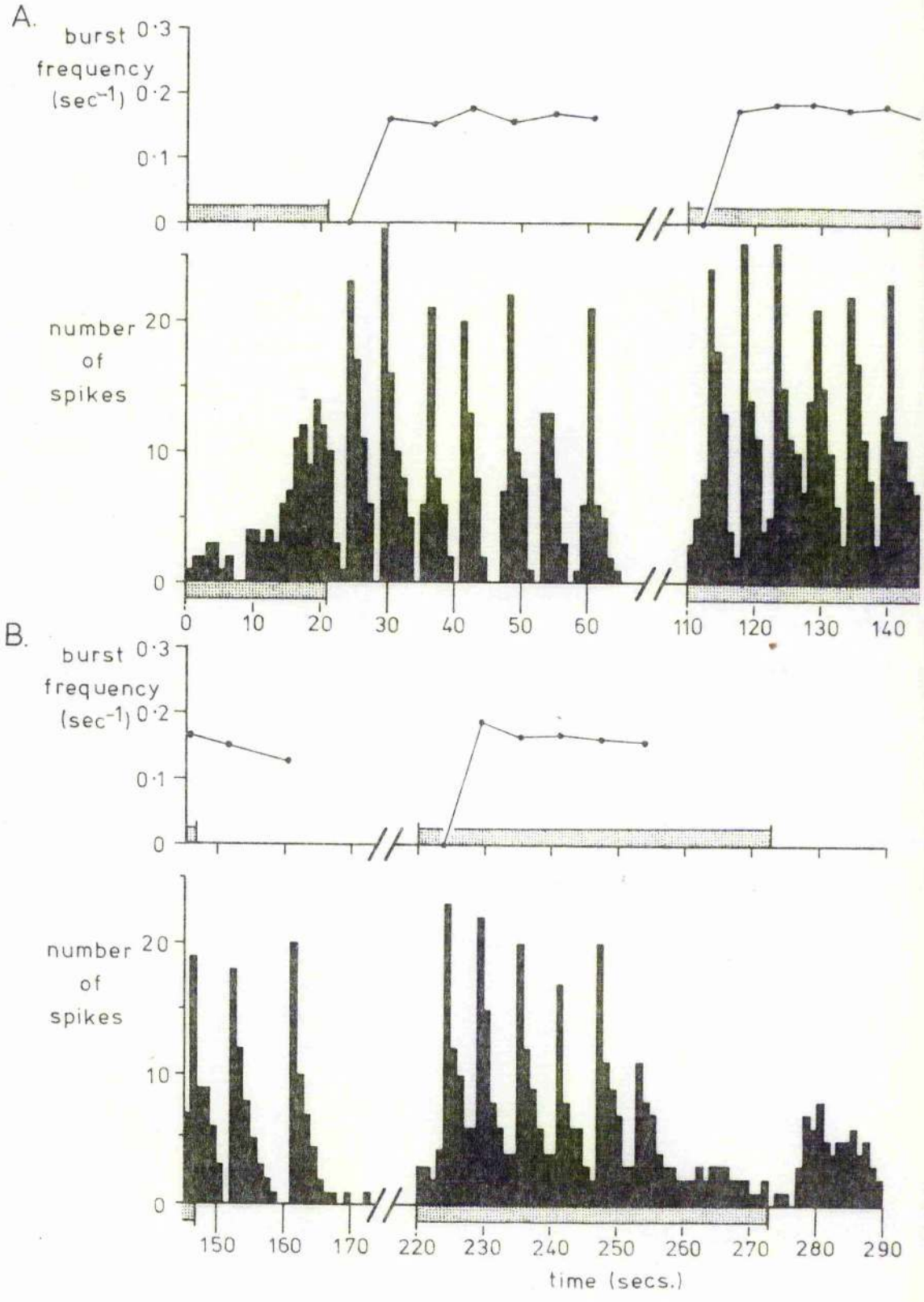

A.
burst frequency (sec⁻¹)
0·3
0·2
0·1
0
number of spikes
20
10
0
0
10
20
30
40
50
60
110
120
130
140
B.
burst frequency (sec⁻¹)
0·3
0·2
0·1
0
number of spikes
20
10
0
150
160
170
220
230
240
250
260
270
280
290
time (secs.)

Figure 48

Trace to show the increase in the frequency of bursting in the s.o.n. on stimulation of the v-p.o.n. Dots indicate the start of each burst and the stimulus artefact is seen clearly.

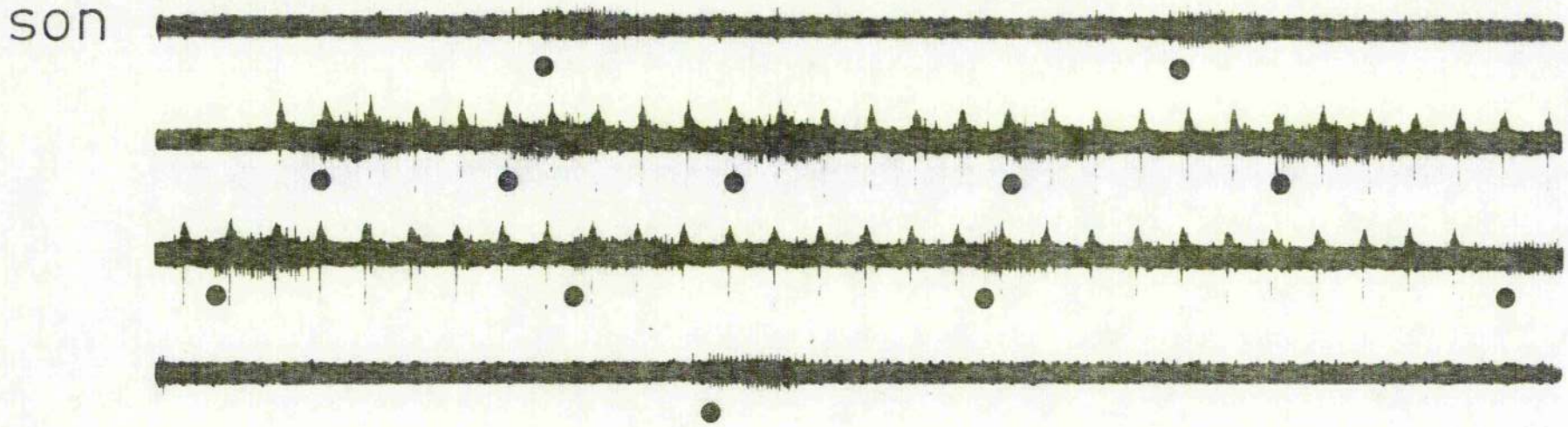

son
300 µv
5 secs.
stimulate - v-pon

Figure 42

Graphs of the increase in the frequency of bursting in the s.c.n. on stimulation of the v.p.c.n. Bars mark the duration of stimulus.

A + B are different experiments.

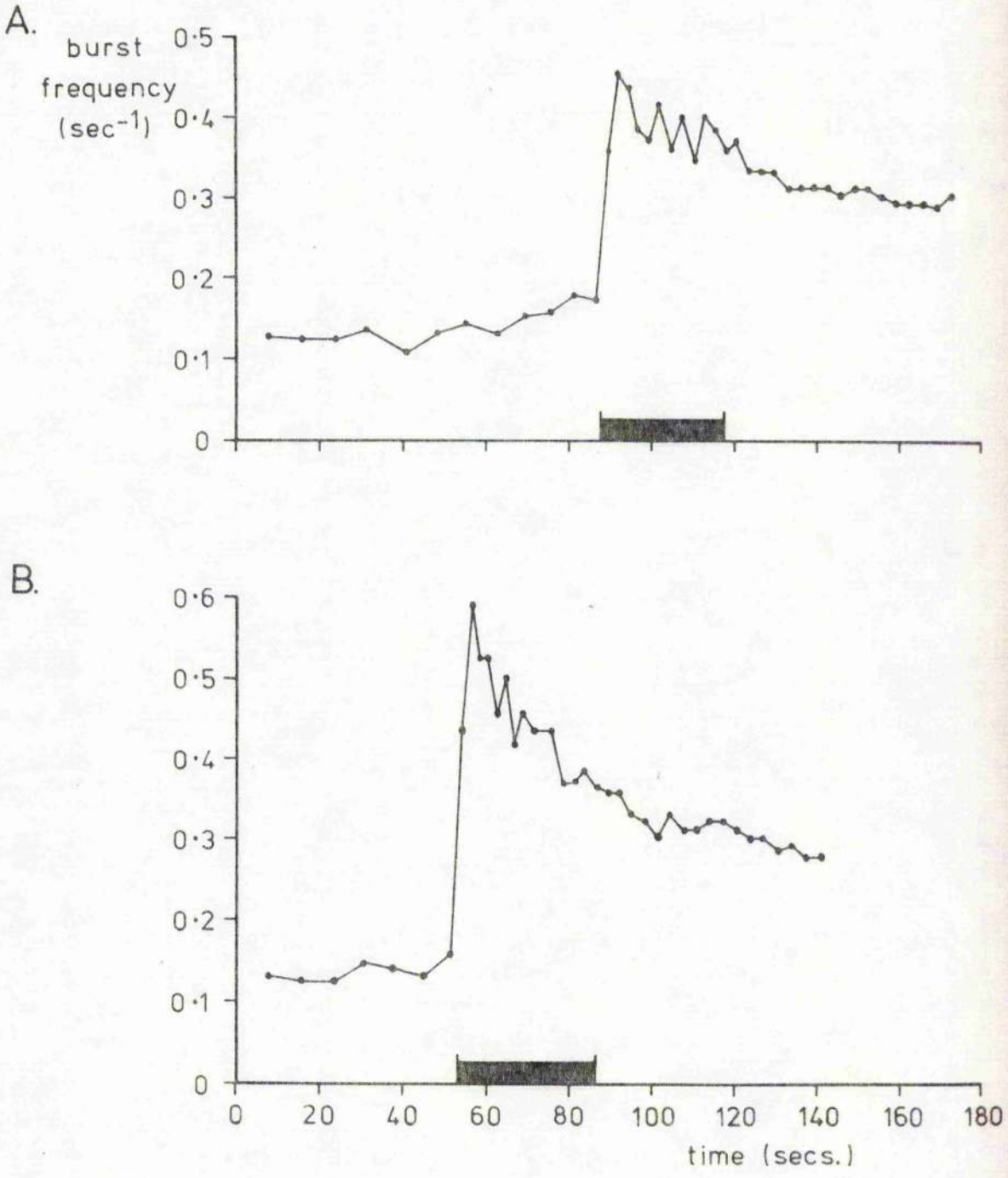
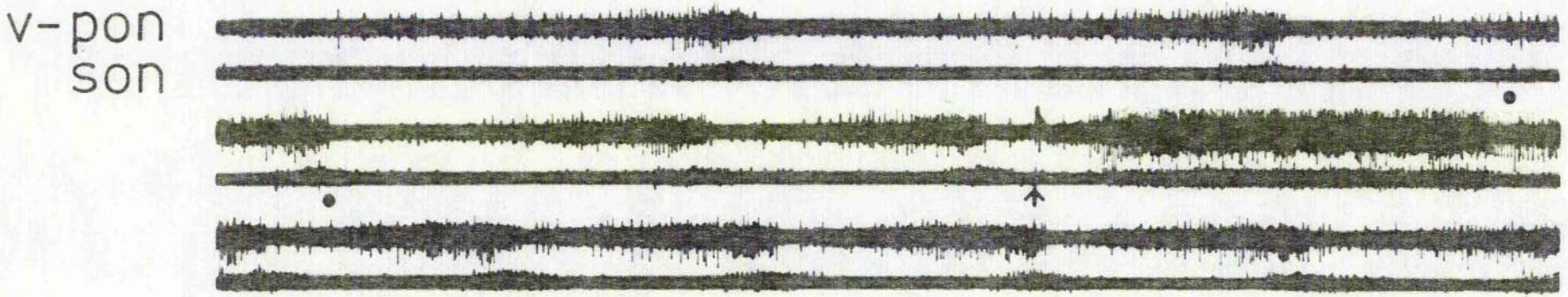

A.
burst frequency (sec^{-1})
0·5
0·4
0·3
0·2
0·1
0
B.
0·6
0·5
0·4
0·3
0·2
0·1
0
0
20
40
60
80
100
120
140
160
180
time (secs.)

Figure 50

Trace to show the increase in the frequency of bursting in the s.c.n. and the v-p.c.n. on application of Mytilus extract to the P.C.S. Dots indicate artefacts associated with positioning of the pipette when some release of the extract may have occurred. The arrow indicates the time of release of the extract.

Also apparent in the v-p.c.n. trace is the shortening of burst length and increase of spike frequency associated with an increase in the burst frequency.



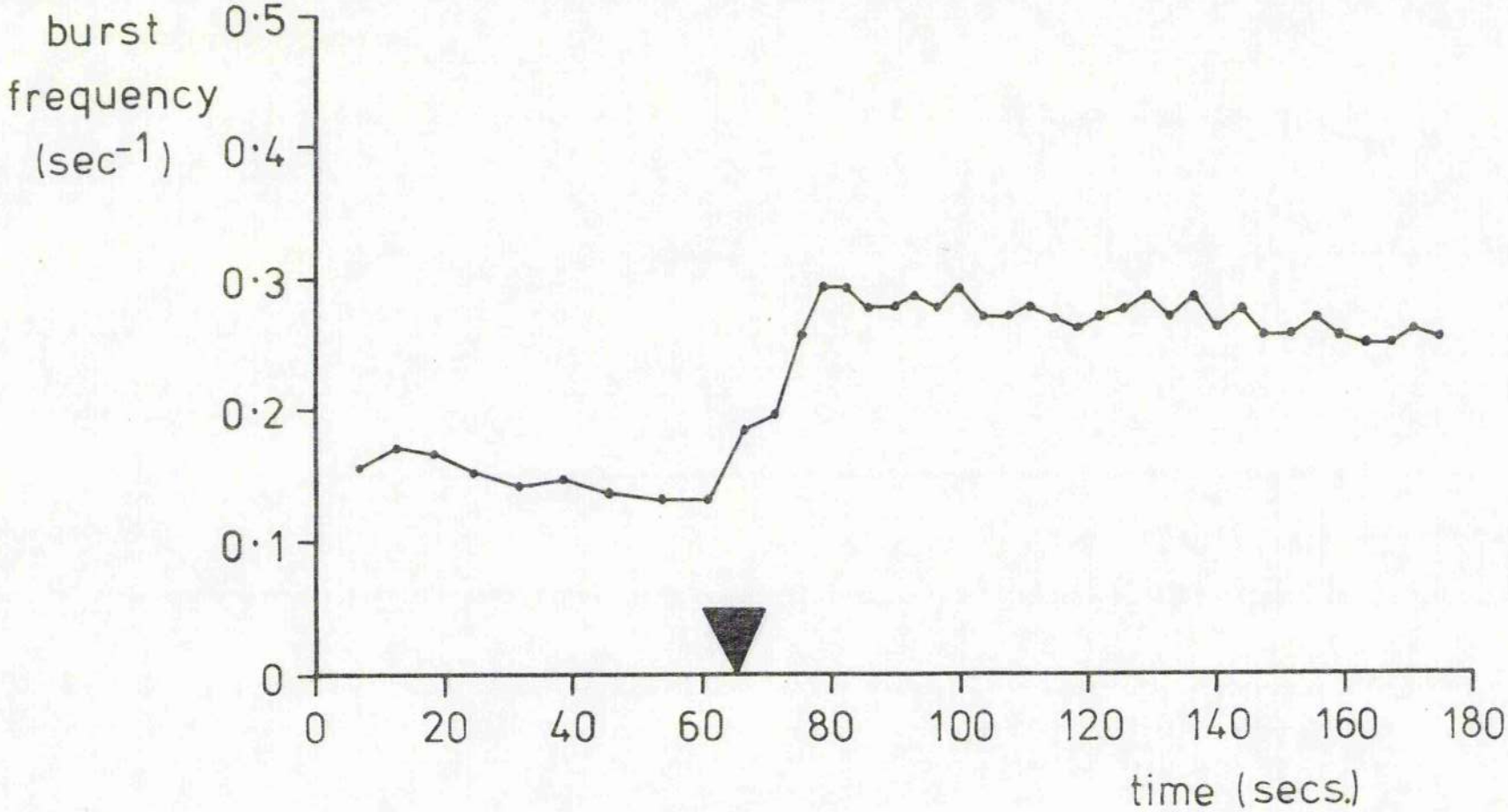
300 µv

5 secs.

stimulus - Mytilus extract

Figure 51

Graph of the increase in the frequency of bursting in the s.c.n. on application of Mytilus extract to the P.O.S. Closed triangle indicates the time of release of the extract.


burst frequency (sec^{-1})
0·5
0·4
0·3
0·2
0·1
0
0
20
40
60
80
100
120
140
160
180
time (secs.)

That is to say application of the extract onto the P.C.S. caused a rapid increase in the a.c.n. burst frequency to a level of c.0.3Hz. which was stable and maintained for several minutes.

(b) Anterior Oesophageal Sensor

During electrical stimulation of the a.o.s.n. two effects on the a.c.n. burst were noticed. Firstly the burst frequency was reduced from whatever its initial level to approximately 0.1Hz. Thus the effect was more dramatic with a higher initial frequency. Secondly, the number of spikes in each burst was reduced. Initially the number of spikes/burst varied around 30; during stimulation this dropped to a variation around 10. On cessation of stimulation the burst frequency and the number of spikes/burst increased, but without reaching their former level. These effects can be seen in the trace of Figure 52 and are graphically depicted in Figure 53.

Attempts to record any sensory activity in the a.o.s.n. came to naught. However, an application of Mytilus extract directly onto the A.C.S. mimicked the effect of electrically stimulating the sensory axons by reducing the burst frequency (Figs. 54 and 55), although there appeared to be no reduction in the number of spikes/burst. The burst frequency could in fact be reduced to zero with continued stimulation (Fig. 55A). To be effective the extract had to be very closely and continually applied to the A.C.S. In Fig. 55B it can be seen that the burst frequency increased towards its original level when the pipette containing the extract was removed but without washing the organ.

4. DISCUSSION

Labral Receptors

The results show that mechanical deformation of the labrum in various ways can produce afferent neuronal activity in the i.l.n. It is probable that this arises from stimulation of the three bilateral groups of sense cells (a, b and c)

Figure 52

Electrical stimulation of the a.c.s.n. with a train of rectangular pulses of width 0.7ms and with an interval of 500ms, showing a decrease in the s.c.n. burst frequency and a reduction in the number of spikes/burst during stimulation.

Dots indicate the start of each burst. The gross stimulus artefact is a result of the proximity of the stimulating and recording electrodes. The amplitude of the stimulatory pulses was not abnormally high.

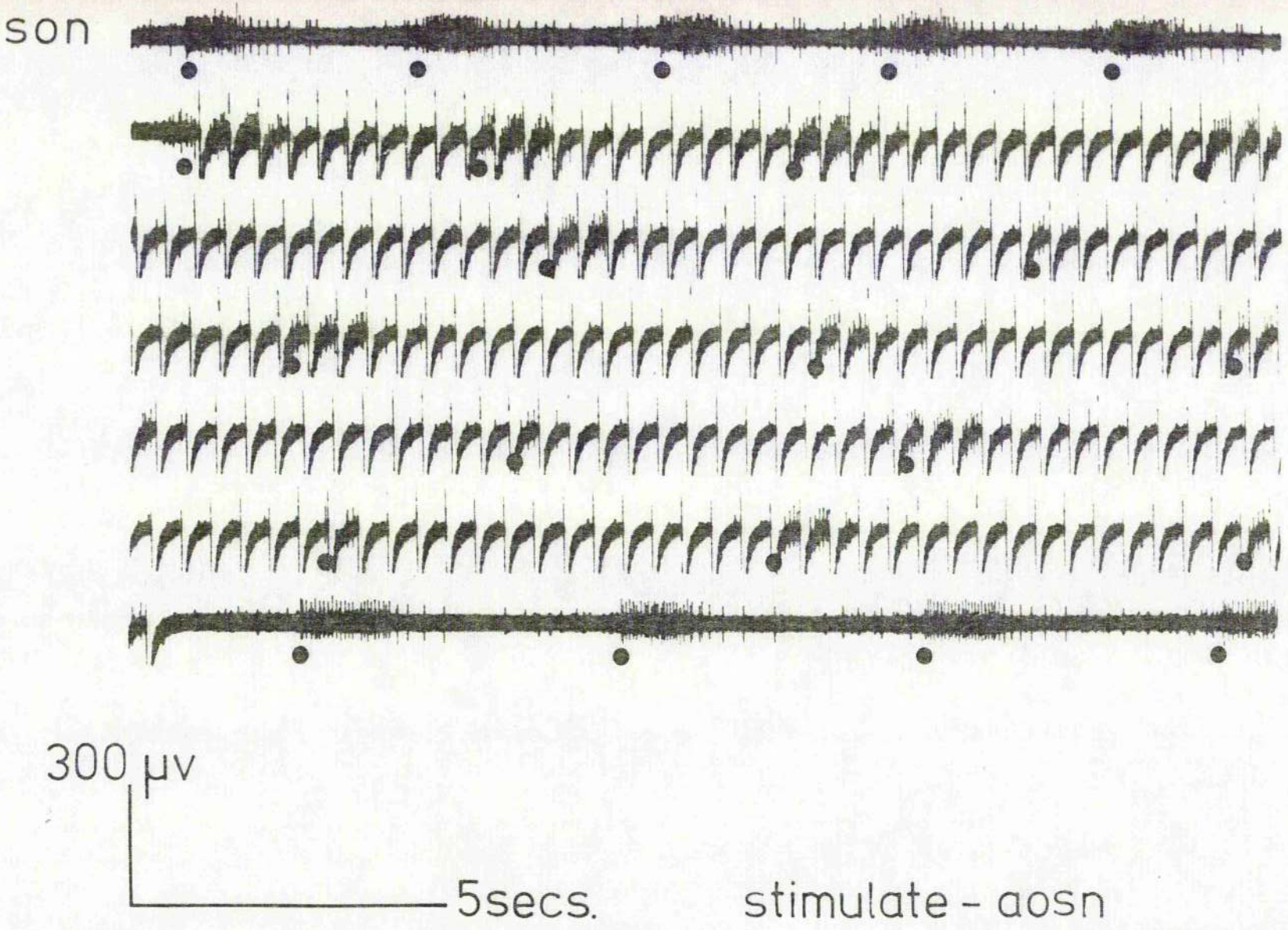
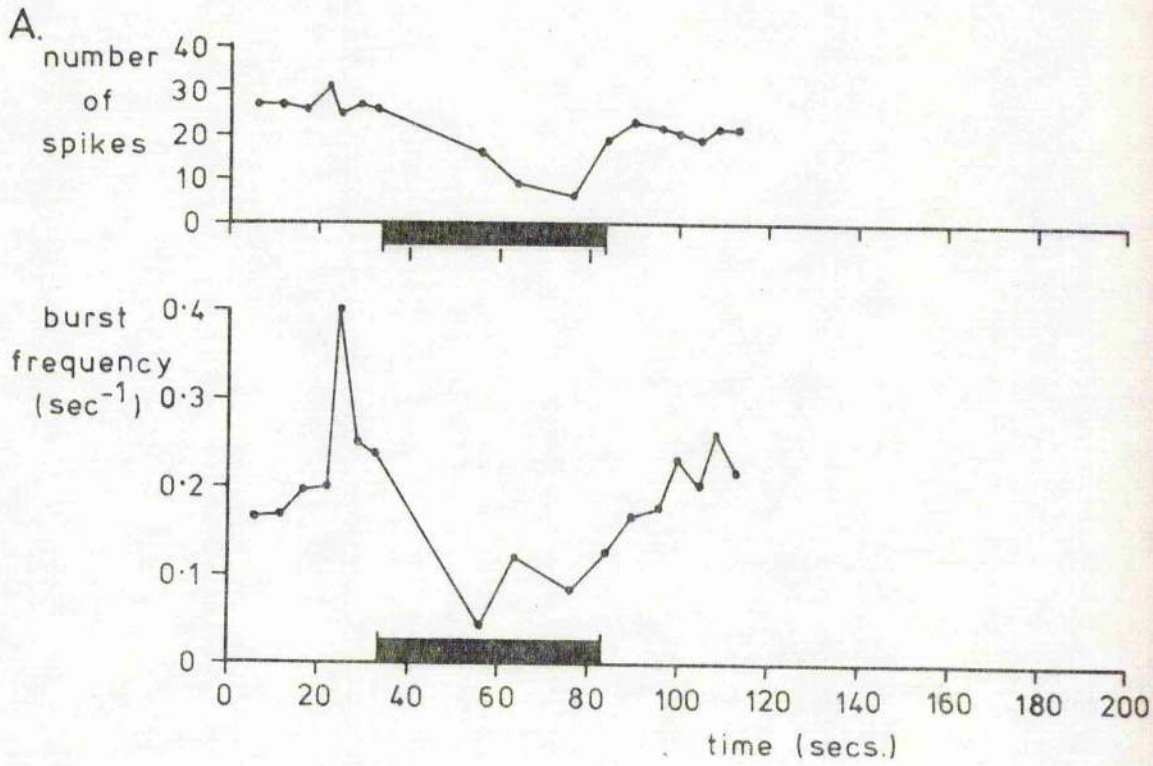

son
300 µv
5secs.
stimulate - aosn

Figure 53

Graphs of the number of spikes/burst and the instantaneous frequency of each s.o.n. burst plotted against the time of occurrence of each burst. This shows the decrease in the number of spikes/burst and the reduction in the burst frequency when the a.o.s.n. is electrically stimulated.

Bar indicates duration of stimulus to a.o.s.n.

A and B are different experiments


A.
number of spikes
40
30
20
10
0
burst frequency (sec^{-1})
0·4
0·3
0·2
0·1
0
0
20
40
60
80
100
120
140
160
180
200
time (secs.)

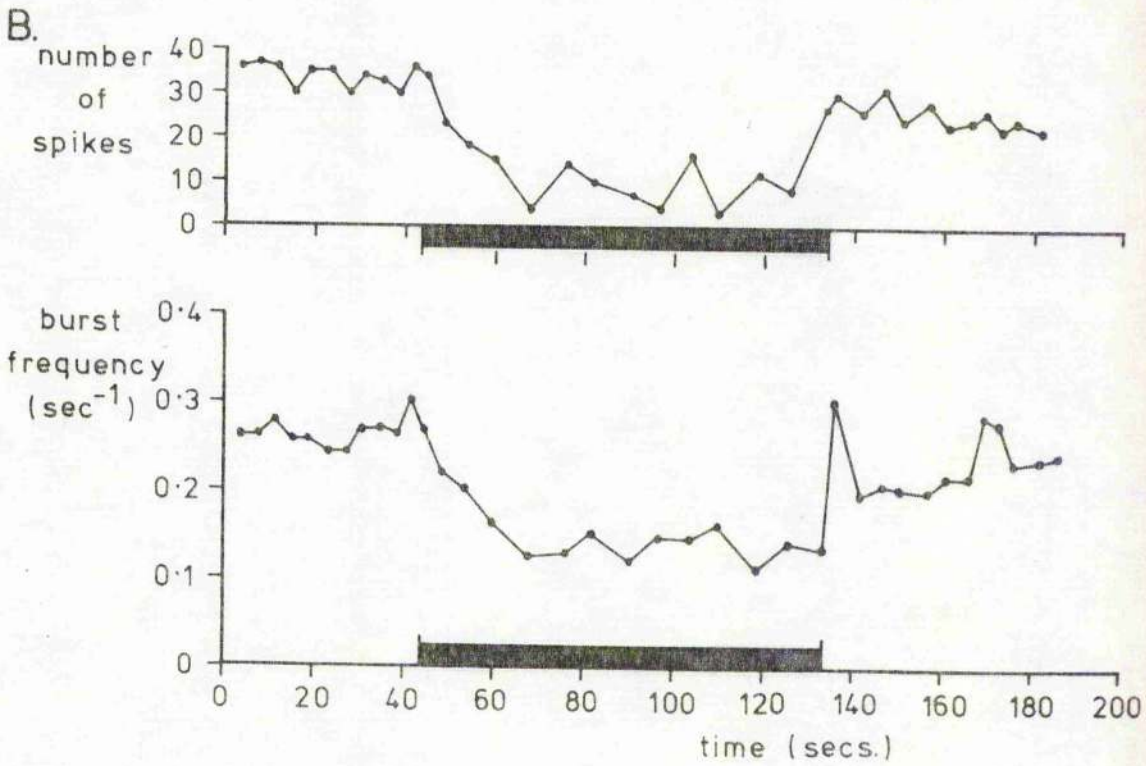

B.
number of spikes
40
30
20
10
0
burst frequency (sec^{-1})
0·4
0·3
0·2
0·1
0
0
20
40
60
80
100
120
140
160
180
200
time (secs.)

Figure 54

Trace showing the decrease in frequency of the s.o.n. burst on application of Mytilus extract to the A.C.S. Time of application indicated by an arrow. Dots indicate the start of each burst.

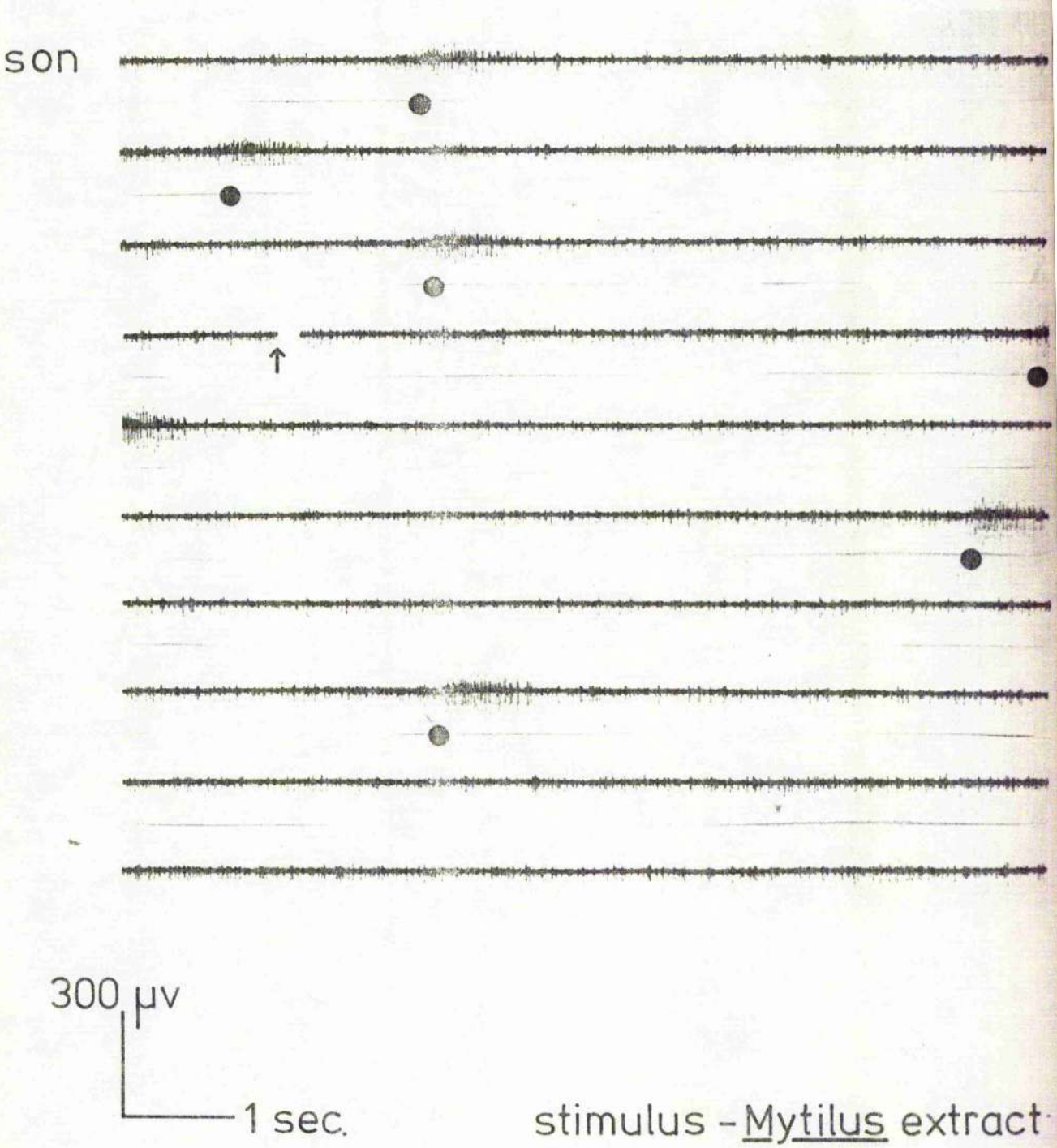
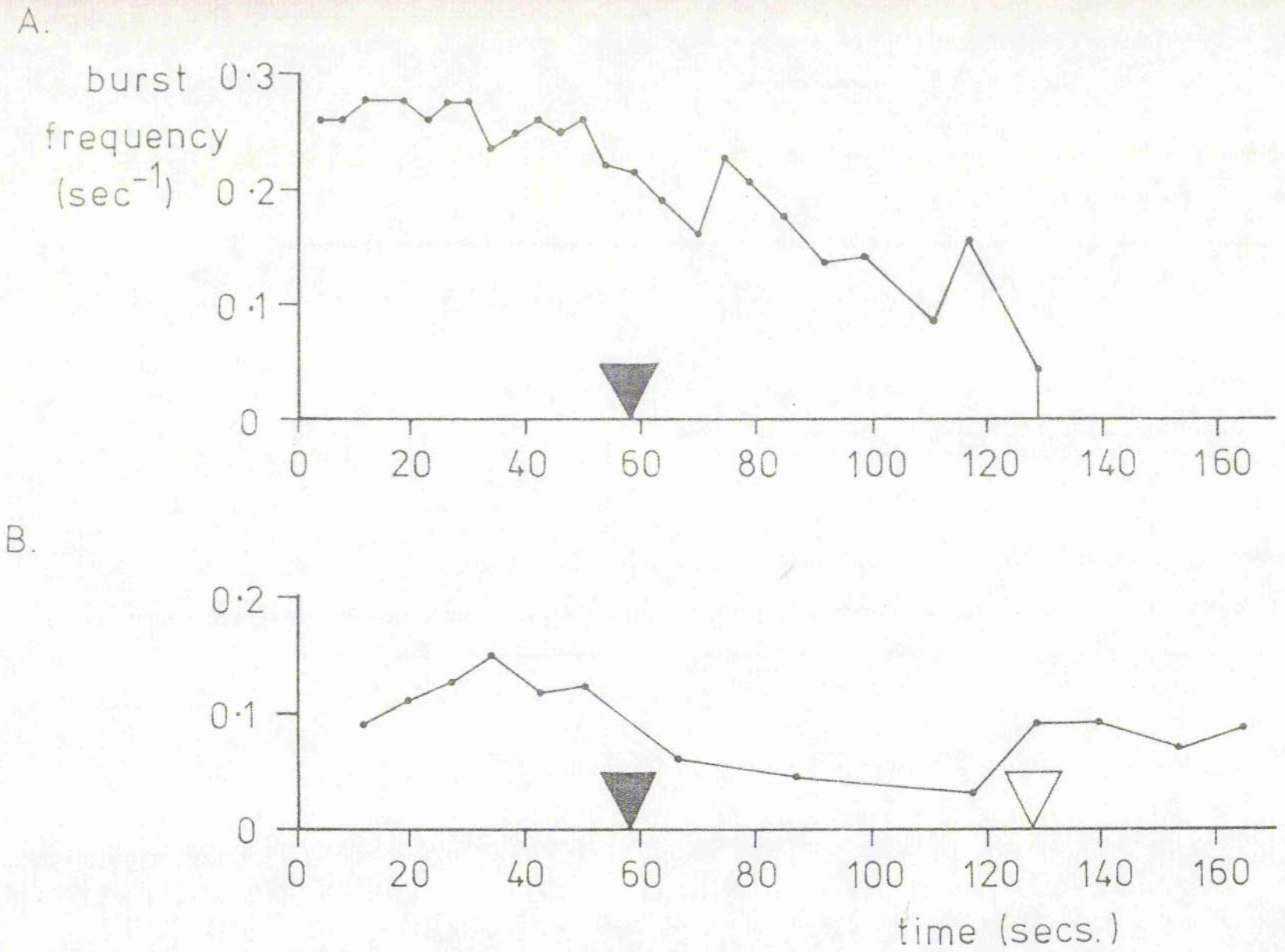

son
300 µv
1 sec.
stimulus - Mytilus extract

Figure 55

Graphs of two experiments showing the decrease in s.o.n. burst frequency on application of Mytilus extract.

A. Closed triangle indicates time of application of extract

B. Closed triangle as above; open triangle indicates removal of pipette containing the extract, without washing the organ.


A.
burst frequency (sec^{-1})
0·3
0·2
0·1
0
0
20
40
60
80
100
120
140
160
B.
0·2
0·1
0
0
20
40
60
80
100
120
140
160
time (secs.)

which have been described, although definitive evidence that this is so is not available. The responses to local stimulation of the soft cuticle of the lobe and lateral walls are typically phasic as described by Dando (1969). Local stimulation of the scutiform sclerite has no effect, however maintained extension of the labrum produces a slowly adapting tonic response (Fig. 43d), while maintained flexion results in a negligible increase in the background activity in the i.l.n. (Fig. 43e). The likelihood is that groups b and c monitor deformations of the soft cuticle of the lobe and lateral walls and group a monitors the overall shape of the scutiform sclerite. Flexion of the scutiform sclerite is unlikely to have very much effect on the dendrites of group a whereas extension will probably stretch them. This may be the potent stimulus for this group. The deformations which are monitored by the labral receptors could be brought about both by the activity of the labral musculature and by the distortive action of food material in the mouth. It has already been shown that changes in the position of the labrum can be registered by the MPR system (Moulins, Dando and Laverack, 1970). This, and the fact that the responses of groups b and c are phasic, suggest that they are primarily involved in obtaining information about the position and movement of food in the mouth, while the tonic responses of group a could register those changes in labral shape which are brought about by the intrinsic labral musculature. More work is necessary before any detailed theory of the normal role of these receptors can be formulated. At the present time it is sufficient to note both their presence and the fact that their output will augment considerably the information being provided to the C.N.S. by other receptors in the area.

No conclusions can be drawn from the fact that in this study no response to chemical stimulation of the labrum could be recorded. Ache (1977) comments on the susceptibility of chemoreceptors to anoxia. This susceptibility coupled with the rather lengthy dissection necessary to gain access to the i.l.n. militates against any chemoreceptors remaining healthy. Furthermore, attempts

to record from the O.S. which are defined as chemoreceptors on morphological grounds also came to naught. However, the O.S. have been shown indirectly to respond to the application of chemicals. Laverack (1975) describes in the nerves innervating the labrum aggregations of nerve fibres of a similar size to described chemoreceptor axons. In addition, although it cannot be confirmed here, Dando (1969) has described small presumptive chemosensory neurons in the labrum. It is possible that further work will reveal chemosensory properties of the labrum.

Control of Oesophageal Peristalsis

(a) Rhythmical Activity

Complex burst patterns have been recorded from the three major nerve trunks arising from each commissural ganglion (s.o.n., v.-p.o.n. and i.o.n.). Nerve branches from the s.o.n. and v.-p.o.n. are responsible for innervating most of the intrinsic and extrinsic musculature of the oesophagus. The branches of the i.o.n. can be considered as innervating the musculature of the mouth (the labrum and the lower limit of the oesophagus). It was found that the rhythmical bursts of the s.o.n. and v.-p.o.n. are similar, whereas that of the i.o.n. is markedly different. Although the fine structure of the i.o.n. rhythm is different, the basic pattern of alternating bursts at the frequency of oesophageal peristalsis is present. It is tempting to speculate that the mouth is innervated by a different set of motoneurons to that controlling the oesophagus; and that this set can be entrained by both the oesophageal and mandibular rhythms. However, this speculation is ill-advised due to the paucity of the results. This lack of data means that little can be concluded about the oesophageal rhythm. In the final event the s.o.n. burst was used solely as an indicator of peristalsis so that the gross effects of O.S. stimulation could be studied.

(b) Initiation of Oesophageal Peristalsis

Intuitive reasoning leads one to the conclusion that effective feeding behaviour is best elicited by chemical stimulation: the release of specific chemicals being a property of potential food material which differentiates it from non-nutritive matter. For example, Lee and Liegeois, (1974) have categorised the chemosensory nerves which are important in food arousal for Pleurobranchaea californica; feeding activity can be induced in Helisoma trivolvis by the application of crushed spinach leaves (Kater and Rowell, 1973); and in Phormia regina there are three groups of chemoreceptors (tarsal hairs, labellar hairs and interpseudotracheal papillae) which are sequentially stimulated to facilitate food ingestion (Gelperin, 1972). Further information can be obtained from Laverack (1974b). Maynard and Dingle (1963) characterised the feeding responses of Panulirus argus to chemical and chemo-tactile stimulation of the antennules, and dactyls of the pereiopods. These responses are similar in Homarus gammarus. The limitation of behavioural studies of this sort is that oesophageal peristalsis cannot be observed in intact, free animals but it is an integral and necessary part of feeding. Thus one cannot determine whether chemical stimulation of the antennules, dactyls and mouthparts is sufficient to induce the total feeding repertoire, or not. Observations of the movements of the labrum during feeding suggest that peristalsis is not initiated until some portion of the food has been inserted into the oesophagus (i.e. small closing movements of the labrum are not evident before or at the beginning of each chewing phase). Thus some internal mechanism, either chemosensory or mechanosensory, must be involved. The present study has shown that electrical stimulation of the v.-p.c.n. and the application of a food extract to the P.O.S. can increase the frequency of the oesophageal rhythm. These facts are taken firstly as indirect evidence for a chemoreceptive function of the P.O.S., and secondly as evidence that the potent stimulus for the initiation of oesophageal peristalsis is chemical stimulation of the P.O.S.

(c) Termination of Oesophageal Peristalsis

Prevention of hyperphagia by some mechanism is essential for feeding animals. To date relatively few such control mechanisms have been studied. Internal inhibitory feedback mediated by gut stretch receptors is documented for Phormia regina (Dethier and Gelperin, 1967); Locusta migratoria (Bernays and Chapman, 1972a, 1973, 1974a); Chorteicetes terminifera (Barton-Browne Moorhouse and van Gerwen, 1975); and Aplysia californica (Susswein and Kupfermann, 1975). In Phormia regina input from abdominal stretch receptors augments the inhibition of feeding mediated by foregut stretch receptors (Gelperin, 1971b). Some measure of control is also provided by the adaptation of chemoreceptors which excite feeding activity (Gelperin, 1971a, for Phormia; Barton-Browne, Moorhouse and van Gerwen, 1975, for Chorteicetes). In Locusta migratoria, as well as adaptation of maxillary palp chemoreceptors, which can be overcome by palpation (Blaney and Duckett, 1975), a mechanism exists whereby the terminal pores of these chemoreceptor sensilla can be closed (Bernays, Blaney and Chapman, 1972). This effect is mediated by a nervous and hormonal pathway of which the first element is foregut stretch receptors (Bernays and Chapman, 1972b). Long term regulation of meal size can be brought about by negative feedback from an increased blood osmotic pressure (Gelperin and Dethier, 1967, for Phormia; Bernays and Chapman, 1974b, for Locusta). However, in most cases, the normal meal size limit is set by foregut stretch receptors irrespective of other conditions (Bernays and Chapman, 1973, for Locusta). The results reported in this chapter suggest that the system which may signal satiation in Homarus gammarus is totally different, being dependent on negative feedback mediated by chemoreceptor excitation.

Electrical stimulation of the a.o.s.n. results in a marked decrease in the frequency of the oesophageal rhythm. It could be argued that this is due to an inhibitory influence of a small group of presumptive stretch receptors

whose axons also can travel in the a.o.s.n. This could over-ride excitatory chemosensory afference. However, chemical stimulation of the A.O.S. mimics the effect. This fact leads to the conclusion that the reduction in the frequency of the oesophageal rhythm is mediated by the A.O.S. Differences can be observed between the responses of electrical and chemical stimulation. These are that the number of spikes/burst is reduced with electrical stimulation and not with chemical stimulation; and that chemical stimulation can terminate the rhythm completely while electrical stimulation has not been seen to do so. If one considers that the presumptive mechanoreceptors may be acting in a similar way to those described co-ordinating the feeding cycle of Helisoma trivolvis (Kater and Rowell, 1973), then an explanation is possible. In Helisoma phasic afferent activity from the mechanoreceptors inhibits the protractor motoneurons of the buccal mass to limit their spike output, and excites the retractor motoneurons to accentuate and regulate their burst. Thus electrical stimulation of the chemosensory axons in the a.o.s.n. would reduce the frequency of the oesophageal rhythm, but the simultaneous stimulation of the mechanosensory axons could be both reducing the spike output of the dilator burst and ensuring that rhythmicity is maintained, albeit at a greatly reduced frequency. The fact that the gross stimulation of the a.o.s.n. is not physiological makes interpretation of the results difficult, and one way of clarifying the situation would be to observe the effect of stimulating single axons separated out of the a.o.s.n.

The structure of the A.O.S. is such that it will only become available for stimulation when the cardiac sac is filled to capacity and the O.C.S.V. is stretched (see Chapter 2). This observation corroborates the hypothesis that the A.O.S. mediates the termination of oesophageal peristalsis by signalling satiation. However, to be effective the response of the A.O.S. to continued stimulation would need to be very slow-adapting. Confirmation that the A.O.S. possesses this property is not available.

Russell ((unpublished) in Selverston, Russell, Miller and King, 1976) has provided evidence that electrical stimulation of a chemoreceptor nerve on the anterior oesophageal wall of Panulirus interruptus provokes a 2-3 fold increase in the frequency of the oesophageal and gastric rhythms. The organ innervated by this chemoreceptor nerve is possibly homologous to the A.C.S. of Homarus gammarus. The fact that this dissertation presents totally contradictor results needs to be explained. Recently Heiss and Norman (1977c) undertook a numerical taxonomical analysis of eleven species belonging to the five infra-orders of the decapod crustacea using homologies between the muscles of the stomatogastric system. Their results are shown in Fig. 56 (their Figs. 1 and 2) Similarity indices representing the percentage of muscles and muscle bundles shared by any two groups were constructed as a similarity matrix (their Fig. 1). The values in this matrix were then computed as a phenogram (their Fig. 2). Firstly the two groups with the highest similarity index were joined, then those with the second highest, and so on. If one group is compared with two others which are already joined then the two groups are considered as one and the similarity indices are averaged. Reference to their Figure 2 reveals that the average similarity between the Palinura and the rest of the Reptantia is relatively low at 61%. Furthermore, Dando and Maynard (1974) have described the organ in Panulirus argus (in the Palinura with Panulirus interruptus), comparing it with the organ in Homarus americanus (in the Astacura with Homarus gammarus) as follows: "In Panulirus argus the arrangement seems to be a little different because although analogous branches of the superior oesophageal nerve occur there does not appear to be a concentration of cells into two distinct groups. The cell bodies are rather more scattered into smaller groups in the general area of the anterior of the oesophagus near the junction with the cardiac sac." These facts indicate that there is no a priori reason for supposing that the functions of the two organs should in any way be similar.

Figure 56

Phenetic analysis of the stomatogastric musculature of several decapod crustacea - from Meiss and Norman, 1977c.

1. Similarity matrix comparing muscles and muscle bundles of the species studied

2. Phenogram illustrating the levels of percentage similarity of several infraorders of decapod crustacea.

Explanation of the derivation of the figures can be found in the text.

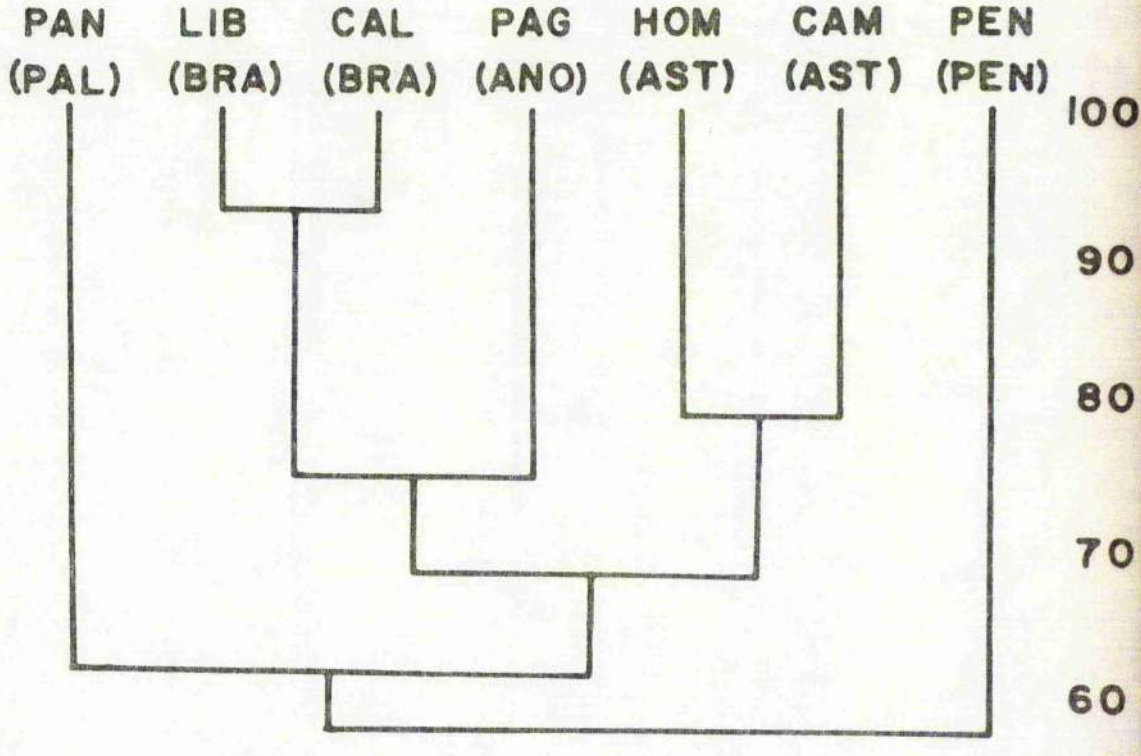
Abbreviations:

Ano	-	Anomura
Ast	-	Astacidea
Bra	-	Brachyura
Cal	-	Callinectes sapidus
Cam	-	Cambarinae (comprising Procambarus clarkii, Cambarus bartonii and Orconectes virilis).
Hom	-	Homarus americanus
Lib	-	Libinia emarginata
Pag	-	Paguroidea (comprising Pagurus pollicaris and Petrochirus diogenes).
Pal	-	Palinura
Pan	-	Panulirus argus
Pen	-	Penaeus spp. (comprising P. duorarum and P. aztecus).

1

	CAL	LIB	PAG	CAM	HOM	PAN	PEN
CAL	X						
LIB	95	X					
PAG	74	79	X				
CAM	68	68	71	X			
HOM	63	63	74	79	X		
PAN	61	61	55	71	55	X	
PEN	52	52	64	62	71	45	X

2



(d) Correlation with Observation of Oesophageal Peristalsis in the Intact Lobster

The small protraction movements of the labrum can be considered as representing oesophageal constriction during peristalsis. Thus the results in Chapter 3 concerning the frequency and duration of labral swallowing activity can be equated with the frequency and duration of oesophageal peristalsis during feeding. It is possible to explain these observations in terms of the integrated activity of the C.S. in the following way:

(a) Starved animals show short duration swallowing sequences with a high initial frequency which rapidly decays. In this case food pushed into the oesophagus stimulates the P.C.S. which give a maximal response to create high frequency bursting in the s.o.n. As the cardiac sac is empty the passage of food through the oesophagus will be rapid. Stimulatory material will be quickly removed from the area of the P.C.S. and rapid decay of the peristaltic frequency will ensue.

(b) Fed, but unsatiated, animals show swallowing sequences of a longer duration, with a lower initial frequency which does not decay as rapidly. This can be explained by the slower passage of food through the oesophagus. The presence of food in the cardiac sac will hinder the entry of more food. Thus the P.C.S. will be stimulated for a longer period resulting in a longer swallowing sequence, but adaptation of the P.C.S. will result in a lower frequency of peristalsis. This effect of adaptation of the P.C.S. can also be seen in those instances when food is present in the oesophagus but is unable to move: peristalsis continues at a minimal frequency and a low amplitude until the food is freed. It is possible that some inhibitory feedback from the A.C.S. may also be affecting the peristaltic rhythm in partially fed animals.

(c) The swallowing activity of satiated animals is erratic, stopping and restarting the sequence at irregular intervals. A full cardiac sac results in inhibitory feedback from the A.C.S. As activity from these organs adapts, peristalsis will be resumed. The movements of the C.C.N.V. during peristalsis may restimulate the A.C.S. similar to palpation of the maxillary palps of Locusta migratoria increasing the amount of chemosensory input reaching the central nervous system (Blaney and Duckett, 1975). This will terminate peristalsis once more. Behaviour like this will continue until the actions of the gastric mill and pyloric filter remove enough food from the cardiac sac to render the A.C.S. unavailable for stimulation. It is plausible to propose that A.C.S. output might act on the gastric and pyloric rhythms to increase their frequency and hasten emptying of the cardiac sac. This would need to be confirmed.

In summary - the results support the hypothesis that initiation of oesophageal peristalsis, and increasing its frequency is controlled by excitatory input from the P.C.S., while slowing and termination of peristalsis is brought about by the adaptation of the P.C.S. and inhibitory input from the A.C.S.

CHAPTER 5

GENERAL DISCUSSION

GENERAL DISCUSSION

The general introduction (Chapter 1) to this dissertation outlines the main aims of the work performed. These were 1) to describe the gross neuromuscular anatomy of the labral/oesophageal complex; 2) to investigate the movements of the labrum during feeding with a view to determining its function; and 3) to describe the anterior and posterior oesophageal sensors and elucidate their role in feeding. The discussions at the end of the experimental chapters (Chapters 2,3 and 4) are detailed considerations of the specific problems. The purpose of the present chapter is to review the results more generally in order to assess the extent to which the objectives were attained, and so that the significance of the results might be appreciated more easily. To achieve these ends a reasonable approach is to consider both how much the data add to existing knowledge and how best the work can be followed up. The divisions of the experimental chapters are based on the experimental techniques employed and for this reason are to a certain extent artificial. Thus links between the results may be overlooked. To circumvent this problem and in an attempt to gain an overall picture of the role in the control of feeding of the structures studied, this chapter will have no divisions except at the end where a model will be presented. This is a model of the contribution of the C.N.S. in the control of oesophageal peristalsis.

Prior to the work reported in this thesis, the labrum of decapod crustaceans had never been subject to detailed investigations of either its anatomy or its function (for a review of the relevant literature see Chapters 2 and 3). This study shows that it is a simple preoral lobe strengthened by several sclerites and invested with a complex musculature which appears capable of controlling a large variety of labral movements. Also, it contains three bilateral groups of mechanoreceptors which respond to local and gross labral deformation. The most recent account of the feeding

sequence of Homarus is that by Barker and Gibson (1977). However these authors virtually ignore the labrum. It is shown here that the labrum moves rhythmically during both the mandibular rhythm (chewing) and the oesophageal rhythm (swallowing). The concept of the labrum and ventral oesophagus serving simply as parts of the mouth is outlined in Chapter 3. The results obtained from electrophysiological recordings lend weight to this argument by showing that the muscles of the main portion of the oesophagus are innervated by a different set of motoneurons (recorded in the v.o.n. and v.-p.o.n.) to that innervating the musculature of the ventral limit of the oesophagus and the labrum (recorded in the i.o.n. and i.l.n.). A possible reason for the presence of a separate control system for the mouth is that it could be entrained by both the mandibular and oesophageal rhythms and thus co-ordinate the transfer of food from mandibles to oesophagus and facilitate a smooth transition from chewing to swallowing. It is concluded that the labrum functions as the anterior portion of the mouth and by moving in the anterior/posterior axis opens and closes the mouth. Further, it is believed that it has a manipulative role mediated by reflexes from the labral mechanoreceptors. These reflexes would need to be characterised electrophysiologically to confirm the function of the receptors.

The gross neuromuscular anatomy of the oesophagus is described in this dissertation. The main difference from existing reports of other decapod crustaceans (see Chapter 1) is that the oesophageal/cardiac sac valve is treated as a separate entity with its own musculature (three paired dilators and the upper limit of the oesophageal constrictor). At this valve two pairs of bilaterally symmetrical chemoreceptors named the anterior and posterior oesophageal sensors are present. The structure of these organs is similar to that of contact (gustatory) chemoreceptors previously described in insects (Moulins, 1968, Moulins, 1971; Cook, 1972). They are classified as

chemoreceptors on morphological grounds and with indirect electrophysiological evidence. The role of the oesophageal sensors in the control of oesophageal peristalsis is outlined in the model below. Also described are two small groups of presumptive mechanoreceptors innervating the oesophagus at the level of the oesophageal/cardiac sac valve.

The foregut of decapod crustaceans can be used as a preparation rich in potential for further work. It will be necessary to make comparative studies of the anatomy of the labral/oesophageal complex in other decapods in the hope of devising a standard nomenclature for the parts and working out the possible homologies. This would add significantly to the work already done by Dando and Maynard (1974), Maynard and Dando (1974) and Heise and Norman (1977a,b,c.). Also, a great deal of work characterising the innervation patterns of the various muscles, and elucidating how they interact could be done. However, perhaps the most interesting and most profitable questions arising from this thesis can be summarised as follows:-

1. Four neuronal rhythms have been described as controlling the foregut of decapod crustaceans (oesophageal, cardiac sac, gastric, pyloric, for a review of the literature, see Chapter 4). This thesis indicates that there may be a fifth (an oral rhythm) which connects mandibular to oesophageal activity. An intracellular search of the commissural and oesophageal ganglia to locate the relevant neurons would be useful. If an oral network is found, a study of the mechanisms whereby it is co-ordinated with the other networks may yield important general results about the control of rhythmical behavioural acts.

2. A similar problem exists for the O.C.S.V. It has been shown that morphologically the valve can be considered as separate from both the oesophagus and the cardiac sac. The question of whether or not this is a true distinction

remains. An electrophysiological analysis of the valve dilators to determine by which, if any, of the known networks they are innervated would resolve the problem. Considering its role to prevent food escape from the cardiac sac, a separate control system for the valve seems improbable.

3. Mechanoreceptors have been described innervating the labrum and there are presumptive stretch receptors at the level of the C.C.P.V. It would be interesting to know the response characteristics and reflex effects of these organs. For the labral receptors this knowledge may indicate the extent of the manipulative role of the labrum. The work described in this thesis has shown that stimulation of the A.C.P. can slow and terminate oesophageal peristalsis. In other animals (see Chapter 4) satiation is signalled by gut stretch receptors. It is probable that the presumptive stretch receptors aid in the control of oesophageal peristalsis either by terminating it when they are continually stimulated or by providing a phasic feedback to co-ordinate the rhythm. Investigations of the influence of feedback from these organs could be useful in extrapolating the results obtained from <u>in vitro</u> preparations to a consideration of the behaviour of the intact animal.

4. A transmission electron microscopal study of the C.P. would be invaluable to compare them with other known chemoreceptors; to determine the number of neurons innervating each nodule; and to try and confirm or deny the presence of terminal pores through the cuticle. Also a knowledge of the response characteristics of the organs would be useful.

5. The principle conclusions of this thesis are that P.C.P. stimulation can initiate and maintain oesophageal peristalsis and that A.C.P. stimulation can slow and terminate oesophageal peristalsis. The discovery that chemosensory afference can terminate a feeding rhythm is significant in

its own right. However a study of the mechanisms whereby such control is exerted would be valuable. Is the afferent activity acting directly on the motoneurons or onto an intervening command element? That a command element can be an integral part of a network has recently been shown (Gillette and Davis, 1977). In Palinurus vulgaris the neurons controlling the oesophageal rhythm are amenable to an intracellular electrophysiological analysis (Moulins and Vedel, 1977). It is probable that this will prove to be the case for Homarus gammarus. Thus this system of the initial and final control of oesophageal peristalsis may afford a good opportunity to study the pathways and mechanisms by which sensory input can control the expression of rhythmical behaviour.

Model of the role of the oesophageal sensors in the control of oesophageal peristalsis

This model (Fig. 57) is presented here to act as the overall conclusion to the dissertation. It is principally concerned with the role of the oesophageal sensors and no provision has been made to include the effects of other receptors (e.g. the presumptive stretch receptors also innervated by the s.o.e.n.). Thus it is understood that stimulation of the C.S. may not be the only means of producing the effects although alone it is sufficient.

Food is broken down by the co-operative action of the mandibles and the 3rd maxillipeds and then presented to the mouth. This presentation has the effect of mechanically stimulating the M.P.R. system and the labral mechanoreceptors to induce the mouth to open. The food is pushed into the oesophagus. As it comes into contact with the P.C.S. the latter are stimulated chemically to effect, by an unknown route, the initiation of oesophageal peristalsis. This results in food entering the cardiac sac. From this point three ways to to continue are possible:

1. If the cardiac sac is not full and food is still being presented, then oesophageal peristalsis continues until the food runs out (2) or the cardiac sac is filled (3).

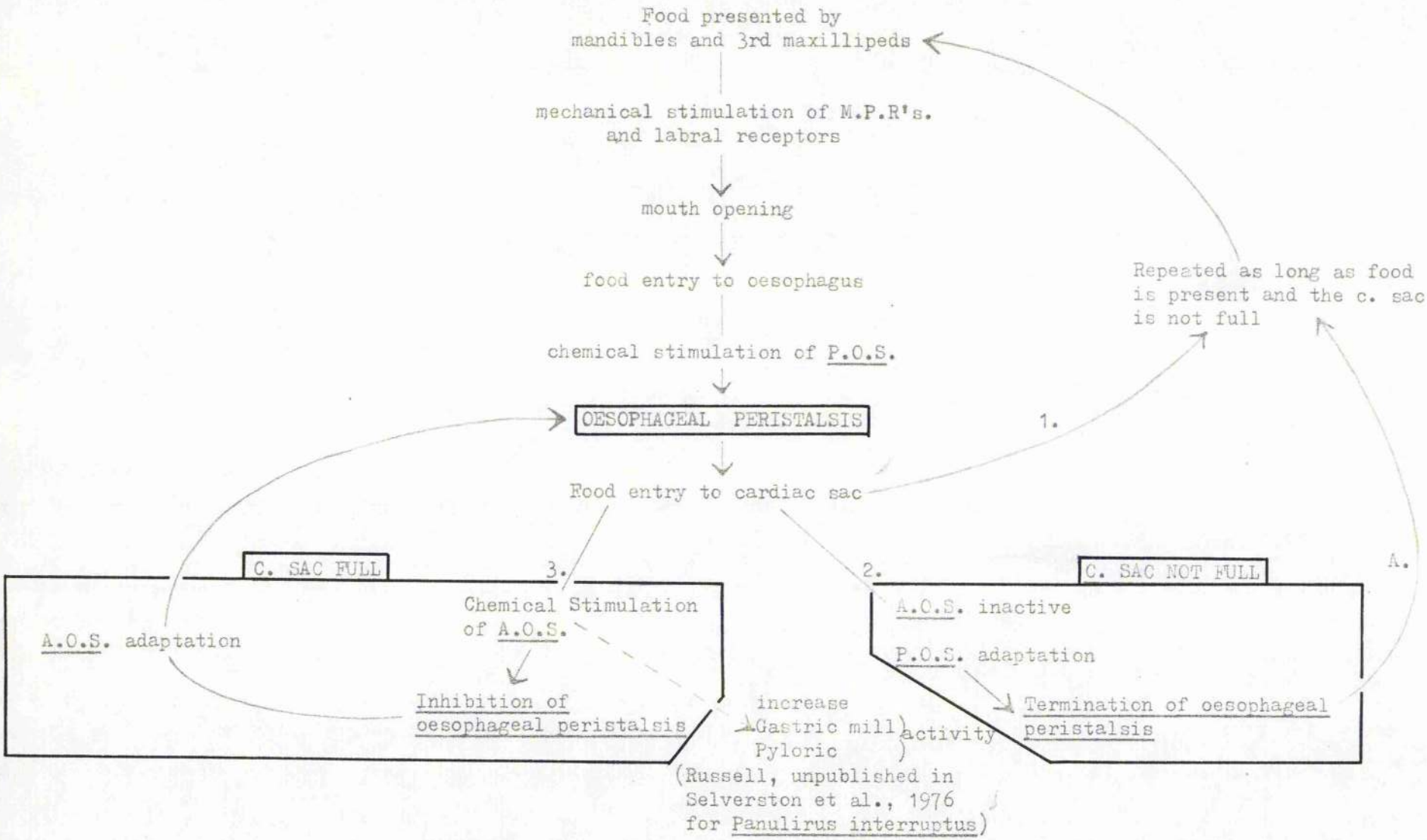
2. If the cardiac sac is not full and the food is finished then the F.O.S. adapt and oesophageal peristalsis terminates until such time as more food is obtained (4).

3. If the cardiac sac is full then the O.C.S.V. will be stretched open allowing stimulatory material access to the A.C.S. Stimulation of the A.C.S. inhibits oesophageal peristalsis. As the A.C.S. gradually adapt oesophageal peristalsis is resumed. In this case it is assumed that movement of the food once more restimulates the A.C.S. (similar to locust maxillary palp palpation (Blaney and Duckett, 1975)). This cycle continues to give irregular bouts of peristalsis until the cardiac sac is emptied by the action of the gastric mill and pyloric filter at which time the sequence returns to (1) or (2). Russell (unpublished in Selvereton, Russell, Miller and King, 1976) has shown that in Panulirus interruptus stimulation of the organ possibly homologous to the A.C.S. has the effect of increasing gastric mill and pyloric activity. It is interesting to speculate that the A.C.S. of Homarus may have a similar effect (dotted arrow) thus contributing to effective feeding. However Russell further showed that stimulation of the organ in Panulirus also increases the oesophageal rhythm, not inhibiting it as is the case for the A.C.S. It was argued in Chapter 4 that there is no a priori reason to suppose that the organs in the two animals should have similar effects, so an affect of the A.C.S. on gastric and pyloric activity must be experimentally demonstrated for Homarus.

Figure 57

Model of the role of the oesophageal sensors in the control of oesophageal peristalsis.

Explanation in text


Food presented by mandibles and 3rd maxillipeds
mechanical stimulation of M.P.R's. and labral receptors
mouth opening
food entry to oesophagus
chemical stimulation of P.O.S.
OESOPHAGEAL PERISTALSIS
Food entry to cardiac sac
Repeated as long as food is present and the c. sac is not full
1.
C. SAC FULL
3.
Chemical Stimulation of A.O.S.
A.O.S. adaptation
Inhibition of oesophageal peristalsis
2.
C. SAC NOT FULL
A.O.S. inactive
P.O.S. adaptation
Termination of oesophageal peristalsis
A.
increase Gastric mill Pyloric activity
(Russell, unpublished in Selverston et al., 1976 for Panulirus interruptus)

SUMMARY

1. The transport of food from the feeding appendages to the cardiac sac in decapod crustaceans has, until recently, received scant attention in the literature. This project was designed to describe the rhythmical activity of the oesophagus and labrum of Homarus gammarus (L.) and to make an exploratory study of its control. It was hoped that the results would yield information of a more general nature concerning the control of rhythmical behavioural acts.

2. The gross neuromuscular anatomy of the labral/oesophageal complex is described.

3. The labrum contains three paired groups of sensory cells which respond to mechanical stimuli.

4. There are two paired sense organs (the anterior and posterior oesophageal sensors) present at the oesophageal/cardiac sac valve. They are classified as contact chemoreceptors on morphological grounds and from indirect electrophysiological evidence.

5. In the resting animal the labrum lies over the opening of the oesophagus. During feeding it can be shown to participate in both the mandibular rhythm and oesophageal peristalsis. The movements of the labrum during the feeding sequence are discussed with reference to its musculature and subsequently are used as an indication of the duration and frequency of peristalsis.

6. Rhythmical bursting neuronal activity can be recorded from the major nerve trunks in the area and acts to set up oesophageal peristalsis. A characteristic burst recorded in the superior oesophageal nerve was used as an indicator of dilation during oesophageal peristalsis.

7. The effects of electrical and chemical stimulation of the oesophageal sensors on oesophageal peristalsis was studied. It was found that stimulation of the posterior oesophageal sensors can initiate oesophageal peristalsis and increase its frequency while stimulation of the anterior oesophageal sensors can slow and terminate oesophageal peristalsis.

8. The results are discussed and suggestions are made for further work in the field.

9. In conclusion a model of the role of the oesophageal sensors during oesophageal peristalsis is presented.

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ABBREVIATIONS

A.F.	-	anterior face of scutiform sclerite
A.L.	-	anterior lobe of oesophageal/cardiac sac valve
ANT.	-	anterior
A.O.S.	-	anterior oesophageal sensor
a.o.s.n.	-	anterior oesophageal sensory nerve
Ap.	-	apical
C..	-	cardiac muscles
C.C.	-	circumoesophageal connective
Ce.G.	-	cerebral ganglia
C.N.S.	-	central nervous system
Co.G.	-	commissural ganglion
Conn.Tiss.	-	connective tissue attachment of oesophagus to cephalic apodeme
C.Sac	-	cardiac sac
C.T.	-	connective tissue
Dors.	-	dorsal
Endo.Sk.	-	1st sternal apodeme of endophragmal skeleton (cephalic apodeme)
Epi.	-	epistoma
Fal.	-	falciform
Fur.	-	furcular
i.l.n.	-	inner labral nerve
i.l.n.(l)	-	lateral branch of inner labral nerve
i.l.n.(m)	-	medial branch of inner labral nerve
Ins.L6	-	insertion of muscle L6.
i.o.n.	-	inferior oesophageal nerve
i.v.n.	-	inferior ventricular nerve
Ky.	-	Kymograph

L..	-	labral muscles
Lab.	-	labrum
L.F.	-	lateral face of scutiform sclerite
Lig.St.	-	ligamentous strap
L.L.	-	lateral lobe of oesophageal/cardiac sac valve
Lo.	-	lobe of labrum
Mand.	-	mandible
Me.blue	-	Methylene blue
Met.	-	metastomal plate
M.ped.	-	maxilliped
M.P.R.	-	mouth part receptor
Nod.	-	nodular
O..	-	oesophageal muscle
O.C.S.V.	-	oesophageal cardiac/sac valve
OCSV..	-	oesophageal cardiac/sac valve muscle
Oes.	-	oesophagus
O.G.	-	oesophageal ganglion
o.l.n.	-	outer labral nerve
o.m.n.	-	outer mandibular nerve
o.n.	-	oesophageal nerve
Oss.	-	ossicle
Parag.	-	paragnath
Ph.Cell	-	photocell transducer
P.L.	-	posterior lobe of oesophageal/cardiac sac valve
p.l.n.	-	postero-lateral nerve
P.O.S.	-	posterior oesophageal sensor
Post.	-	posterior
Ret. para	-	retractor paragnatha
Sc.	-	sclerite
Scut.	-	scutiform
S.G.	-	suboesophageal ganglion
S.L.R.	-	supra-labral ridge
s.o.n.	-	superior oesophageal nerve
St.n.	-	stomatogastric nerve
Vent.	-	ventral
v.-p.o.n.	-	ventral-posterior oesophageal nerve

PUBLICATIONS

Part of this thesis has been published in the following:

ROBERTSON, R.M. and LAVERACK, M.S., (1978), Inhibition of oesophageal peristalsis in the lobster after chemical stimulation. Nature, 271, 239-240.