A Thesis presented for the degree of

Master of Science entitled:-

"A study of structure and function in the stomatogastric nervous system of the locust species Schistocerca gregaria Forsk. and Locusta migratoria migratorioides R. and F. and the lobster Homarus gammarus (L.)."

by

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SUPERVISOR'S CERTIFICATE

I certify that Janet Dando has fulfilled the conditions laid down under Ordinance No. 51 of the University Court, St. Andrews, and is accordingly qualified to submit this thesis for the degree of Master of Science.

DECLARATION

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

VITAE

I was educated at The Red Maids' School, Bristol and subsequently at Bristol Technical College. I attended the University of St. Andrews as an undergraduate from October 1964 to June 1968 and graduated in Zoology. The work reported in this thesis was carried out between August 1968 and August 1969.

CONTENTS

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SUMMARY

1. This study set out to provide ultrastructural information on the ingluvial ganglion of locusts (Schistocerca gregaria F. and Locusta migratoria migratorioides R. and F.) and the stomatogastric ganglion of the lobster (Homarus gammarus (L.)), that might be of use in physiological investigations of these ganglia and associated nerves, aimed at elucidating the mode of functioning of the foregut and its nervous system. The two ganglia, which occupy similar positions on the foregut, are parts of the arthropod stomatogastric nervous system.

2. The demonstrated presence of biogenic amines in both the arthropod central nervous system and the visceral nervous system of decapod crustaceans prompted a search for their presence in the ingluvial ganglion of S. gregaria. Such amines may act as nervous transmitter agents or play a role in physiological regulation.

3. An understanding of visceral control requires a detailed knowledge of nervous anatomy and activity. The introduction accordingly contains descriptions of gut innervation and reviews some of the physiological work performed on the arthropodan heart and gut (the most thoroughly investigated of the viscera and both reportedly innervated by the stomatogastric system). Also reviewed in the introduction are some of the pharmacological studies of the heart and gut which may be of importance when trying to establish the nature of visceral neurohumoral agents. Table 1 summarizes what is known of the presence in and action on the heart and gut of compounds known to act as transmitter agents in other situations, and of compounds extracted from other arthropod organs such as corpora cardiaca and pericardial organs.

4. The evidence obtained from ultrastructural studies and by the histochemical fluorescence method (Falck 1962; Falck et al. 1962) indicate the likely presence

in the two ganglia investigated, of dopamine and/or noradrenaline.

5. Biologically active substances currently considered likely arthropod transmitter agents are discussed, as is the lack of information concerning visceral neurotransmitters. Evidence suggests that biogenic amines may be involved in visceral control in arthropods and while their transmitter status is not established is sufficient to warrant further investigation of such a possibility. The suggestions for further work indicate the lines of research which might be followed in attempting to establish a transmitter and/or hormonal role for such amines in the gut.

I INTRODUCTION

1. GENERAL

In recent years there has been much interest in the physiology of invertebrate nervous systems (see Bullock and Horridge 1965) as investigators have realized the frequent advantages they possess over vertebrate systems for studies of neuronal structure and function (e.g. see Kerkut 1967; Cottrell and Laverack 1968). Invertebrates provide a greater diversity of morphological types and frequently several favourable preparations are found within an animal (e.g. arthropods).

Many of the recent important studies on invertebrate nervous systems have utilized the phylum Arthropoda which has supplied the majority of best-known physiological preparations such as the crustacean MRO, P.D. organ, chordotonal organs, insect flight control system, etc. (Both chemical and electrical events within and between cells are being studied).

One part of the arthropod nervous system which perhaps has received less attention than is warranted is the stomatogastric nervous system (SNS) which offers some excellent targets for structural and physiological analyses of nerves, ganglia and neuromuscular interactions. This system in arthropods innervates mainly the anterior alimentary canal (including the heart), though nerves may extend to the midgut. The stomatogastric system comprises a collection of nerves and small ganglia and has its main connections to the tritocerebrum and anterior ventral ganglia. Its location implies involvement in control of the processes of ingestion, passage and digestion of food and there is evidence (see later) for this role. While there may be a degree of autonomy in certain of the ganglia (e.g. see Clarke and Grenville 1960) there is almost certainly overriding central control (Bullock and Horridge 1965).

Particular advantages which the stomatogastric nervous system possesses

over the central nervous system (CNS) for anatomical and physiological studies, include the following:-

(i) it is often more readily accessible by dissection

(ii) the ganglia and nerves are in general much simpler than those of the CNS (i.e. fewer cells and fibres) and may often control relatively simple, often stereotyped activities (e.g. ossicle movement in the decapod stomach, valve opening and closing in insects, swallowing, etc.) For these reasons the possibility exists of making a complete analysis of the smaller ganglia and nerves (see Maynard 1966) which probably will provide information fundamental to an understanding of function in more complex central ganglia.

This thesis is concerned with a ganglion of the arthropod stomatogastric system - the ganglion situated on the posterior foregut of insects and decapod crustacea. The main part of the investigation concerns the paired ingluvial ganglia of two locust species, Schistocerca gregaria F. and Locusta migratoria migratorioides R. and F., and a smaller part the median unpaired stomatogastric ganglion of Homarus gammarus (L.) (= Homarus vulgaris Milne-Edwards).

The main aim of the work was to provide anatomical (where required) and histological information on the ganglia and some nerves, which could form a basis for future physiological work on the system.

Virtually nothing is known of visceral neuro-neuronal or neuromuscular transmitters in arthropods. Many substances have been isolated from arthropod whole body and nervous tissues (see Treherne 1966), some of which are known transmitter substances. They include acetylcholine (Ach), adrenaline (A), noradrenaline (NA), dopamine (DA), γ-aminobutyric acid (GABA), L-glutamate, and 5-hydroxytryptamine (5-HT).

Studies of the fine structure of the ingluvial and stomatogastric ganglia revealed the presence of numerous dense-cored vesicles in fibres of

the neuropile, resembling elementary neurosecretory granules described in a wide variety of vertebrate and invertebrate species, and those vesicles believed to be the intraneuronal storage sites of catecholamines (CA's) and 5-HT.

It is now possible, using a variety of experimental techniques which include those of Falck and Hillarp (Falck 1962; Falck et al.1962), and Wood (1966) not only to locate monoamine-containing cells, identify the amine and estimate its quantity, but also to recognise the amine-storing organelles within a given cell (Welsh 1970). Monoamines have been shown to have a widespread distribution in the nervous systems of many invertebrate groups (see Rude 1969 for references) and to occur frequently in the visceral nervous system.

Since monoamines have been demonstrated, using the Falck and Hillarp histochemical fluorescence method, in the arthropod CNS (Frontali and Norberg 1966, Periplaneta; Elofsson et al. 1966, Astacus); in the nerve supply to the hindgut (Elofsson et al. 1968, Astacus); and in the stomatogastric ganglion of Homarus gammarus (Osborne and Dando 1970), the technique was used in this study in an attempt to demonstrate the presence of monoamines in the ingluvial ganglion of Schistocerca gregaria.

While biogenic monoamines can clearly have diverse roles in physiological regulation (see for example: Biogenic Amines as Physiological Regulators, ed. J.J. Blum, Prentice-Hall Inc. N.J.1970), so that their presence in nervous tissue does not necessarily imply a transmitter function, nevertheless it is the possible transmitter role of monoamines in the arthropod visceral nervous system which will be considered in the discussion.

The remainder of this introduction is a survey of some previous work on the arthropod heart and gut which may be relevant to an understanding of gut function and visceral regulation.

2. ARTHROPOD VISCERAL NERVOUS SYSTEM

INSECT GUT

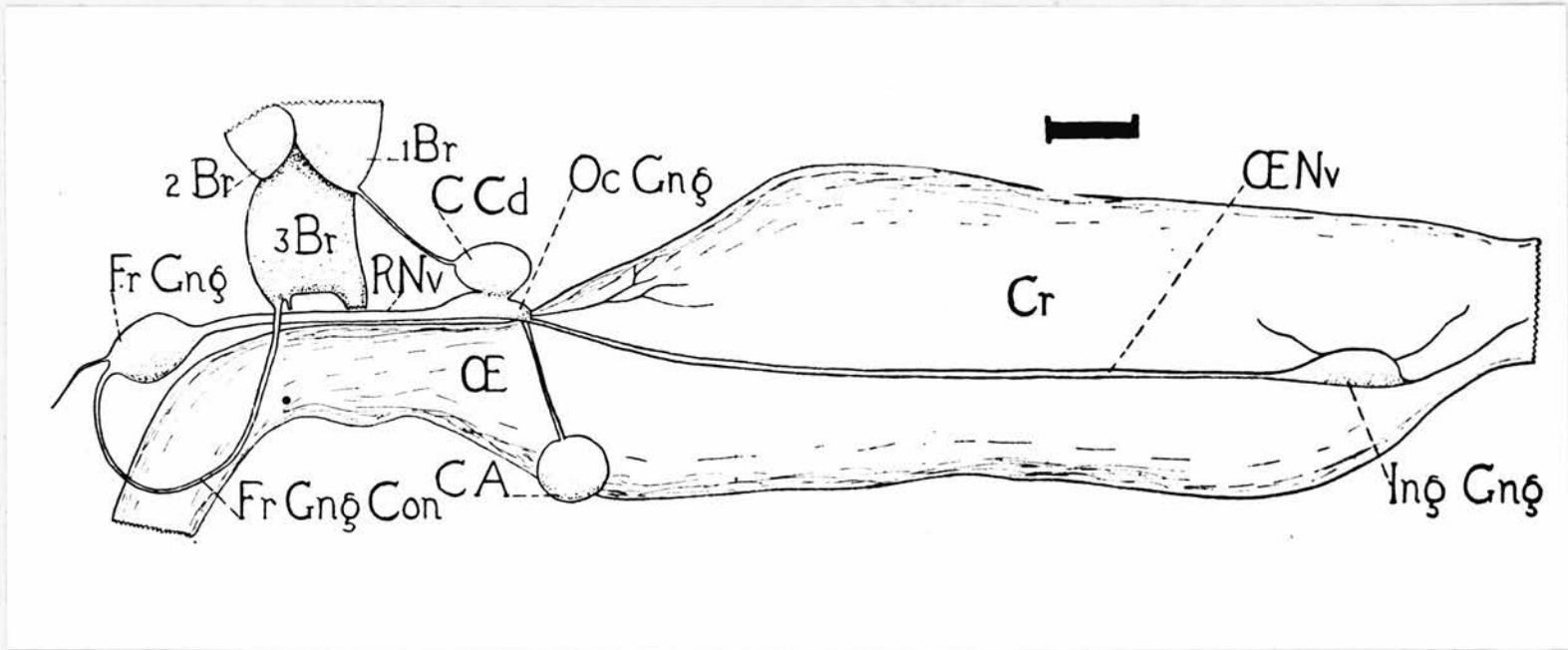
(a) Anatomy

The insect foregut includes buccal cavity, pharynx, oesophagus, crop and gizzard (proventriculus). The proventricular valve separates fore and midgut. The midgut often possesses digestive caeca anteriorly. Malpighian tubules are located at the junction of the midgut with the hindgut.

The foregut, heart and parts of the midgut are innervated from the stomatogastric (or stomodeal) nervous system (for a discussion of the relative merits of the two names see Bickley 1942; Campbell and Burnstock 1968), and the hindgut by nerves from the last one or two abdominal ganglia, branches of which also supply the Malpighian tubules. As far as is known the nerves are mixed motor and sensory and form a plexus over much of the alimentary canal (Bullock and Horridge 1965). Multipolar sensory cells are distributed over the gut and bipolar sensory neurones with a peripheral process which runs inward through the wall are abundant anteriorly, e.g. in the pharynx (Bullock and Horridge 1965). Stretch receptors have been described in the insect foregut (Gelperin 1967; Möhl 1969; Rice 1970).

The following description of the main nerves and ganglia of the SNS is taken from Bullock and Horridge (1965). The most anterior ganglion, the median frontal ganglion (see Fig. 1) lies at the centre of a bridge formed by a pair of frontal ganglion connectives of the tritocerebrum and in some orders is also connected to the tritocerebrum by a median nerve. Posteriorly from the frontal ganglion a median recurrent nerve runs under or through the brain along the dorsal surface of the oesophagus to the median hypocerebral ganglion which is joined by short nerves to or is more intimately connected to the paired glandular corpora cardiaca. Median or paired nerves

Figure 1. **Diagram showing the left lateral aspect of the stomatogastric** nervous system of Schistocerca americana (Drury) and its relationship to **the foregut (after Bickley 1942).** 1, 2, 3 **Br - divisions of the brain;** CA - corpus allatum; C Cd - corpus cardiacum; Cr - crop; Fr Gng - **frontal ganglion; Fr Gng Con - frontal ganglion connective; Ing Gng -** ingluvial ganglion; Oc Gng - occipital ganglion (hypocerebral); OE - **oesophagus;** OE/NV **- oesophageal nerve;** RNv **- recurrent nerve.**


1Br
2 Br
3Br
C Cd
Oc Gnç
ŒNv
Fr Gnç
RNv
Cr
Œ
Fr Gnç Con
C A
Inç Gnç

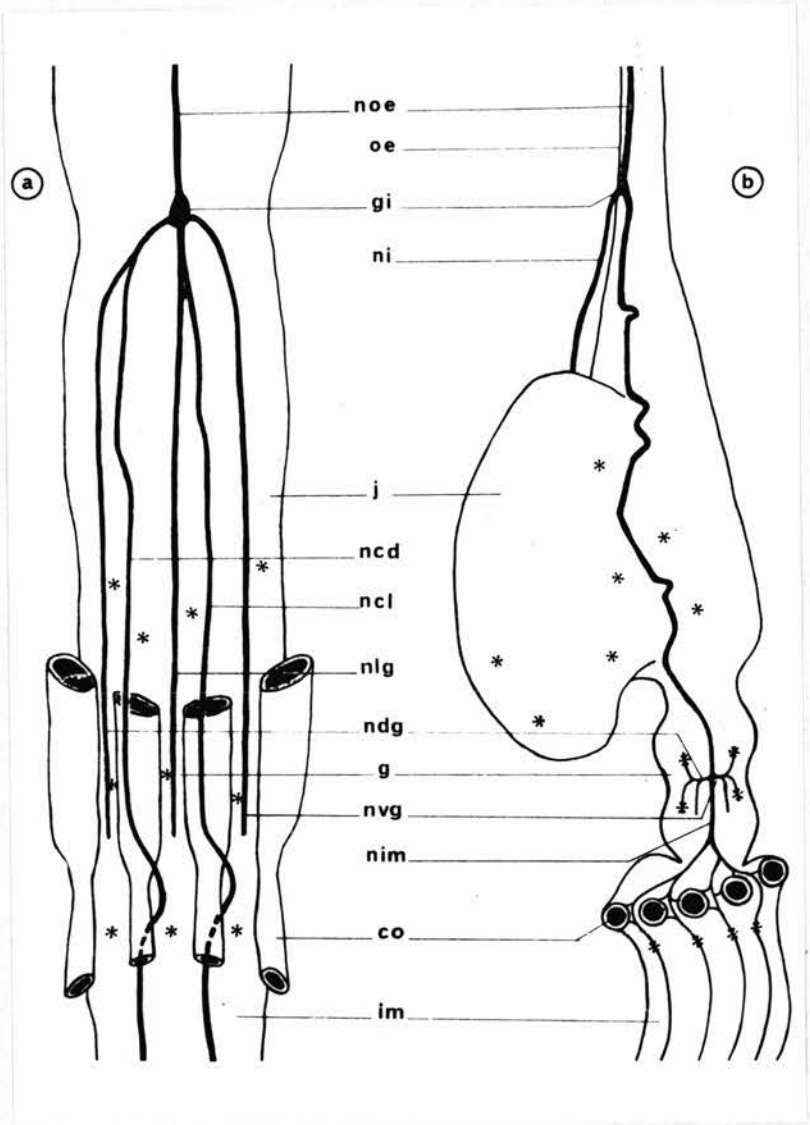
or both leave the hypocerebral ganglion posteriorly and run back over the foregut to the ingluvial ganglia (fused in some insects e.g. cockroaches).

In Schistocerca (Fig.1) the paired posterior nerves of the hypocerebral ganglion are the inner (not labelled) and outer oesophageal nerves, the former branching along the dorso-lateral oesophageal wall and the anterior wall of the crop and sending fibres to the aorta, and the latter terminating in the ingluvial ganglia situated postero-laterally on the crop. Several nerves from the ingluvial ganglion (here called ingluvial nerves) innervate the posterior foregut and the midgut. In S. gregaria the ingluvial ganglion has a central neuropile almost completely surrounded by a cortex of neurone somata (approximately 20 x 25 um) and glial cells. Three main nerves leave the ganglion to innervate the posterior part of the crop, the gizzard and the midgut (Fig.2 from Dando et al. 1968), one ventral, one lateral and one dorsal. The ventral nerve (n_{vg}) runs to the gizzard, the other two soon divide, each giving rise to a gizzard nerve (nlg and ndg) and a caecal nerve (ncl and ncd).

In locusts there are six caeca at the anterior end of the midgut. The three gizzard nerves from each ganglion run along the crop and gizzard to the bases of the anterior caecal lobes but do not extend to the midgut. The two caecal nerves from each ganglion run along the outer sides of the two lateral anterior caecal lobes and onto the midgut, passing under the posterior lobes of the same caeca. Not shown in Figure 2 is a prominent anastomosis between the two ventral gizzard nerves which may provide a pathway for fibres to pass from one side of the gut to the other, allowing movements of the two sides to be co-ordinated.

Thus ten main nerves from the paired ingluvial ganglia innervate the posterior foregut and the midgut. Six are gizzard nerves innervating the crop, gizzard and anterior caecal lobes, while the four caecal nerves are

Figure 2. Diagram of the posterior region of the stomatogastric nervous system in (a) Schistocerca gregaria and (b) Blabera craniifer (from Dando et al. 1968). co - caecum; g- gizzard; gi - ingluvial ganglion; im - midgut; j - crop; ncd - dorsal caecal nerve; ncl - lateral caecal nerve; ndg, nlg, nvg - dorsal, lateral and ventral gizzard nerves; noe - oesophageal nerve; oe - oesophagus. The asterisks indicate the distribution of sensory cells, those on the anterior midgut marking the position of sensory cell groups referred to in the text.


a
b
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primarily nerves of the midgut. The caecal nerves have been traced almost to the end of the midgut and possibly extend further. They run parallel on the midgut and their lateral branches are generally few and small. The exceptions are larger branches near the caeca which extend to groups of neurones situated on the midgut longitudinal muscle bundles where they begin to diverge after passing from the gizzard between the caeca (see Fig. 2). In these sensory cell groups an average of twelve neurones per group were stained in methylene blue preparations. Each group may be innervated by branches from more than one caecal nerve.

Sensory neurones are numerous on the surface of the crop and gizzard but few on the anterior caecal lobes and midgut and are apparently absent on the posterior caecal lobes. They also occur along or within nerves and at nerve junctions. The sensory neurones are multipolar and are probably Type II sensory cells (Pringle 1961) with branched unspecialized dendrites ending in or on connective tissue. Such a cell is shown in Figure 6.

Unlike all other insect ganglia those of the SNS have not been shown to contain neurosecretory material (NSM)(Uvarov 1966) when stained with classical neurosecretory stains (e.g. see Delphin 1963; Strong 1966; Dogra and Ewen 1970). With the same staining techniques, however, NSM has been demonstrated in the oesophageal nerves (Thomsen and Møller 1959; Strong 1966; Dogra and Ewen 1970).

There is little published work on the ultrastructure of the SNS Chanussot (Chanussot et al. 1969) has investigated the ingluvial ganglion and nerves of Blabera craniifer with the electron microscope and the results have been compared with those from S. gregaria. While Normann (1965) reported that the hypocerebral neurones of Calliphora appear to be ordinary nerve cells with no special morphological signs of secretory activity, he did note that the Golgi complexes of the cells may concentrate a substance to produce dense

granules resembling neurosecretory granules. Only a few such granules occurred in the cells. Smith (1968) did not report the occurrence of such granules in the hypocerebral neurones of Carausius.

Axons containing NSM (much or all of which may originate in brain neurosecretory cells) leave the corpora cardiaca en route for a variety of target organs which include the corpora allata, salivary and prothoracic glands, heart and visceral muscle (see Smith 1968). Smith described axons running through the sheath surrounding the midgut which contain opaque neurosecretory droplets. He believes these axons include motor units of the SNS which terminate on the gut muscles. The Malpighian tubules (Maddrell 1969) and the rectal papillae (Smith 1968) also have a neurosecretory innervation and Brown (1967) found large dense granules (ca .200 nm in diameter), in addition to normal synaptic vesicles, in axonal elements making neuromuscular junctions with the rectal longitudinal muscles of Periplaneta americana.

(b) Physiology

The movements of the insect gut are complex (see Davey 1964) and there may be some variation in the role and degree of specialization of particular gut regions in different insect groups, depending on their mode of feeding.

The stomatogastric nerves regulate the intake of food and the movements necessary for digestion. Bullock and Horridge (1965) indicate resemblances of the SNS to the vertebrate autonomic system: - it has sensory neurones directly connected to the CNS and others which end in small peripheral ganglia and it has motor fibres to the anterior alimentary canal of both central and peripheral origin. Most of the gut movements are centrally co-ordinated and the stomatogastric ganglia, unlike the heart ganglion, do not appear to be locally autonomous (Bullock and Horridge 1965).

The frontal ganglion has suitable connections to be a motor centre co-ordinating local sensory input with premotor excitation from the CNS via the frontal commissure and is on the sensory pathway from gut to brain (Bullock and Horridge 1965). The ganglion has been shown to be important in some insect species in control of swallowing, regulation of crop-emptying and foregut tonic contraction for example (see Clarke and Grenville 1960; Campbell and Burnstock 1968) but in others it is apparently non-essential for the maintenance of foregut activity and the more peripheral ganglia may exert control. Engelmann (1968) found, by severing the recurrent nerve just behind the frontal ganglion or the oesophageal nerve behind the hypocerebral ganglion one hour after feeding, that food release from the crop of Leucophaea maderae was halved. If the same operations were made 1 - 12 days before feeding this inhibition of crop-emptying was not observed though the animals generally ate less than controls. Engelmann suggested that the ingluvial and proventricular ganglia probably assumed autonomous control of the proventricular valve, in the absence of the frontal ganglion. Removal of the brain after feeding had no effect on the rate of crop-emptying.

Davey and Treherne (1963b) found that severance of oesophageal or ventricular (ingluvial) nerves virtually halted crop-emptying in Periplaneta americana and in a preparation with a contracting proventriculus, stopped valve movements. A connection between the frontal ganglion and the CNS was apparently non-essential to normal crop-emptying.

Preliminary experiments involving electrical stimulation "of the nervous pathway between pharynx and proventriculus" (Davey and Treherne 1963b) resulted in valve-opening. In Blaberus giganteus Cook et al. (1969) showed that stimulation of the oesophageal nerve innervating the crop region caused a slow type of graded contraction in the longitudinal muscles. Stimulation

of the peripheral end of a sectioned outer oesophageal nerve in S. gregaria (either with the bilateral nerve sectioned or intact) resulted in contractions of the crop and gizzard slightly more complex than those occurring spontaneously (Dando 1968). Stimulation of the central end of the cut nerve (with the bilateral nerve intact) evoked a similar contraction of the crop and gizzard. These experiments all indicate motor control of the foregut from centres other than the ingluvial ganglion but the fibres stimulated could be of central origin or derive from cells in the more anterior stomatogastric ganglia.

Gelperin (1967) described foregut stretch receptors in Phormia, located in a nerve branch connecting the recurrent nerve and the foregut. He suggested that the receptors supply the brain with information about the duration and extent of foregut peristalsis (a measure of the fullness of the crop). If receptor input was eliminated by sectioning the recurrent nerve in front of or behind the brain (Dethier and Gelperin 1967) feeding behaviour was not inhibited in the normal way and hyperphagia resulted. Gelperin implicated the receptors in the satiety phenomenon but Moulins (1970) has pointed out that in Diptera as in other insects, foregut proprioceptors are much more numerous than Gelperin's work would suggest and therefore the origin of the arrest of feeding is probably not yet established. Rice's (1970) study of stretch receptors in the cibarial pumps of tsetse and blowflies showed that simulated pumping evokes bursts of action potentials in the cells, with each neurone having a discharge frequency proportional to the degree of indentation of the anterior cibarial wall. Stimulation of different wall areas resulted in different firing patterns. The receptors may monitor and perhaps control the rate and type of pumping. Multiterminal nerve cells in the foregut of crickets have been

shown to be stretch-sensitive (Möhl 1969). The spontaneously active cells respond phasotonically to stretch.

In S. gregaria Clarke and Grenville (1960) found that the ingluvial ganglion plays an important role in control of foregut movements and is autonomous in its effects since movements continued normally when the ganglion was isolated from more anterior regions of the SNS. The hypocerebral ganglion was found to influence the rate of relaxation of gut musculature but the effect was not mediated through the ingluvial ganglion and the inner oesophageal or recurrent nerves may be involved. Section of the ventricular (ingluvial) nerves stopped rhythmic gut movements (Clarke and Grenville 1960; Dando 1968).

Until now there has been little electrophysiological investigation of motor control of the foregut by the SNS. However, Möhl (personal communication) is working on this problem in the cricket Acheta and has recorded burst activity (probably motor) in a sidebranch of the nerve connecting hypocerebral and ventricular (ingluvial) ganglia.

There is little information concerning the control of mid-and hindgut by nerves and hormonal control may be important (see Campbell and Burnstock 1968).

(c) Pharmacology endocrinology ← Poor little

Ach at low concentrations stimulates contraction of the whole alimentary canal (see Davey 1964) and the effect is potentiated by some anticholinesterases. Cholinesterase has been demonstrated in the intestine of Periplaneta (see Davey 1964). Freeman (1966) found that the fore- and hindgut of Locusta migratoria were unresponsive to Ach, but in contrast Cook et al. (1969) reported that both innervated and denervated foreguts of Blaberus giganteus were sensitive to 5×10^{-6} M Ach, showing an increase in amplitude and frequency of contractions.

Indolalkylamines such as tryptamine, 5-HT and 5, 6 - dihydroxytryptamine reportedly excite the insect hindgut (see Davey 1964) but Holman and Cook (1970) observed no remarkable effect of 5-HT on the hindgut of Leucophaea maderae. Roach foregut responds to 10^{-9} M 5-HT (threshold), being more sensitive than either heart or hindgut (Brown 1965). It responds with an increase in frequency and amplitude of phasic contractions and at higher concentrations such contractions are replaced by a tonic contraction. Freeman (1966) found both the foregut and particularly the hindgut of Locusta migratoria to be excited by 5-HT and the hindgut of Periplaneta americana to be excited by 5 -HT and tryptamine. The foregut of Blaberus giganteus responds to 5-HT with an increase in frequency and amplitude of contractions (Cook et al. 1969).

DA was found to have an excitatory effect upon the hindgut of L. migratoria (Freeman 1966) but Holman and Cook (1970) reported that DA and to a lesser extent NA, inhibited the spontaneous hindgut activity of Leucophaea maderae. At 8×10^{-5} g/ml DA caused nearly complete arrest of myogenic and neurogenic activity within three minutes. An inhibitory material in gut extracts produced a hindgut response similar to DA. In the foregut of L. migratoria DA had no effect on a quiescent preparation though in an active preparation 2µg/ml decreased the amplitude and frequency of contractions whereas 4µg/ml produced small contractions of longer duration (Freeman 1966). Innervated and denervated foreguts of Blaberus giganteus increased their amplitude and frequency of contractions in the presence of NA (Cook et al. 1969). A and NA had no effect on quiescent foregut preparations of L. migratoria but both produced an increase in amplitude and frequency of contractions in a spontaneously active foregut (Freeman 1966). Factor S (a biogenic amine of uncertain structure found

in several species of cockroach including Blaberus giganteus excited innervated foreguts of this species but inhibited denervated preparations (Cook et al. 1969).

A table of this for would be useful

L - glutamic acid was found to have an excitatory effect upon the foregut of B. giganteus (Cook et al. 1969), with denervated foreguts being more sensitive than innervated preparations. Both L - glutamic acid and L - aspartic acids have been isolated from extracts of viscera and nervous system of leucophaea maderae and Periplaneta americana (Holman and Cook 1970) and the hindgut of L. maderae was found to be sensitive to both amino acids. Both amino acids caused excitation of the hindgut in the form of phasic contractions but L - aspartic acid did not effect the same degree of desensitization of the neurally evoked response as did L - glutamic acid. Holman and Cook believe this could suggest that some type of glutamate receptor exists in insect visceral muscle and that if L - glutamic acid were the excitatory transmitter then applied high concentrations of the amino acid would flood the post-synaptic receptor sites and block the action of the natural transmitter in neurally evoked contractions.

Denervation of the foregut and removal of the ingluvial ganglion had no effect on the reaction of the foregut to 5-HT, Ach or NA (Brown 1965; Cook et al. 1969) and it would seem that their observable effects are not mediated by the ganglion. This leaves as alternative sites of action, intrinsic neural networks, neuromuscular junctions, pacemaker cells and the transverse tubular structures of the excitation-contraction system of visceral muscle (Cook et al. 1969).

The contraction rate of Malpighian tubules is increased by A, 5-HT and 5, 6 - dihydroxytryptamine (see Davey 1964). 5-HT also stimulates secretion by the tubules, known to be under hormonal control, but 5-HT

is not identical with the diuretic hormone (Maddrell et al. 1969).

Extracts of arthropod organs such as the corpora cardiaca and allata, the brain and suboesophageal ganglion, are known to have an excitatory effect on the gut (see Davey 1964). Davey (1962) found that extracts of the corpora cardiaca were effective on the hindgut only when the anterior colon (containing argentaffin cells) remained attached. He suggested that the active component of extracts caused the release of a substance by the argentaffin cells (proposed to be an indolalkylamine) which subsequently activated the nerves of the intestinal plexus. Colhoun (1967) doubts the existence of double hormone action however, and thinks it more likely that an active agent from the corpora cardiaca either stimulates the gut directly or if a second hormone is involved it is not 5-HT or an indolalkylamine. Extracts of the corpora cardiaca can increase excretion through the Malpighian tubules and reduce reabsorption in the rectum of S. gregaria (Mordue 1969).

The distribution of NSM-bearing axons to various regions of the gut (see earlier) may mean that pharmacologically active substances within the droplets, released from nerve endings, act as neurotransmitters or local hormones and may affect the functioning of muscles or in the midgut, secretion of digestive enzymes also (Smith 1968). In the rectal papillae the neurosecretory axons may possibly release an anti-diuretic hormone (Gupta and Berridge 1966a, see Smith 1968). In the Malpighian tubules the axons may liberate a diuretic hormone but if they do it is known that they are not the major source of this hormone (Maddrell 1969) and therefore may have some other effect on the tubules.

Brown (1967) extracted a substance (s) from the fore-and hindgut of Periplaneta americana which caused a slow graded contraction in the

longitudinal muscles of the cockroach proctodeum. The specific activity of the substance was highest in nerves innervating the gut - for stomodeal and proctodeal nerves, 25 and 10 times higher respectively than in the viscera they innervate, suggesting a neural origin for the gut factor. The concentration in visceral nerves was up to 150 times that in thoracic peripheral nerves innervating somatic muscles. Brown could not identify the substance with peptides P_1 or P_2 (from the corpora cardiaca), 5-HT, Ach, A, NA, GABA or glutamate and apparently did not consider DA. He proposed that the factor acts as an excitatory neuromuscular transmitter in the longitudinal proctodeal muscles and probably in other visceral muscles of the cockroach.

Recently Holman and Cook (1970), investigating the pharmacological properties of excitatory neuromuscular transmission in the hindgut of cockroaches, provided evidence that at present, L - glutamic acid (glutamate) is the best prospective chemical mediator at the visceral excitatory myoneural junction (see above). Using extraction procedures nearly identical to those of Brown, they obtained from crude extracts of gut and nerves three components with an excitatory effect on the hindgut of Leucophaea maderae. Of these one was believed to be a neurohormone, and the other two were identified with L - glutamic and L - aspartic acids. Holman and Cook believe the substance described by Brown to be a mixture. Though the two amino acids affected the hindgut similarly, the failure of L - aspartic acid to effect the same degree of desensitization of the neurally evoked response as L - glutamic acid, led Holman and Cook to propose the latter as the more likely candidate for the excitatory transmitter. The presence of almost three times as much L - glutamic acid in hindgut as in foregut suggested also a possible fundamental

physiological difference between the two.

CRUSTACEAN GUT

(a) Anatomy

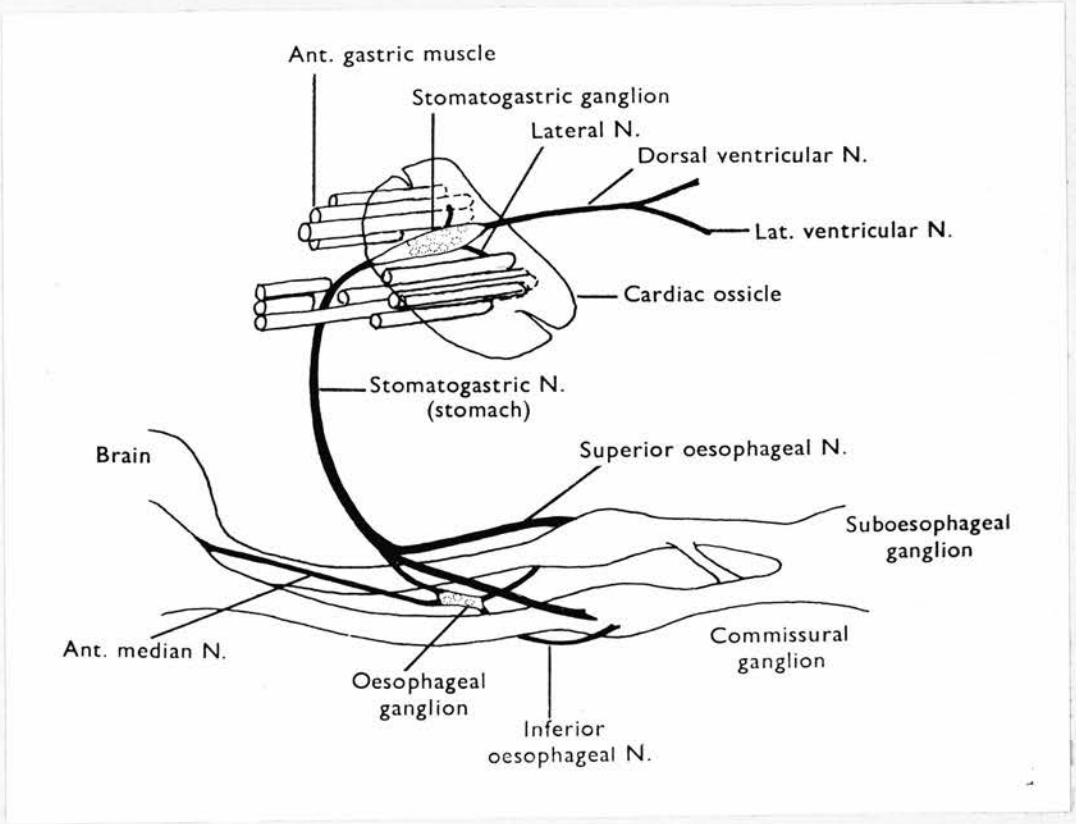
In the Decapoda Reptantia (which includes lobsters and crabs) the general structure of the gut is as follows. A short vertical oesophagus opens into the sac-like cardiac stomach (containing the gastric mill) which leads to the pyloric stomach. The pyloric stomach is elaborated into a press and filter for controlling the size of food particles passing to the midgut and digestive diverticula for digestion and absorption. The midgut leads to the hindgut, terminating at the anus.

The gut musculature is striated. That of the foregut is the most highly developed and here the muscles are mainly bilaterally arranged. These muscles move the foregut to bring about ingestion, trituration, filtering and food passage. The stomach muscles are of two kinds, intrinsic (constrictors) and extrinsic (dilators, with their distal attachments to the carapace), and must operate in a well-defined sequence for the foregut to function properly. They are innervated by mixed motor and sensory nerves of the SNS. There is no known inhibitory innervation of the foregut.

The following description of foregut innervation is taken from Bullock and Horridge (1965) and Dando (1969).
Figure 3 should be consulted.

On each circumoesophageal connective lies a commissural ganglion from which arise the following main nerves:-
(i) and (ii) the superior and inferior oesophageal nerves which meet with those of the opposite side at the median elongate oesophageal ganglion (on the anterior outer wall of the cardiac stomach and giving rise to some thin motor nerves to the visceral muscles),

Figure 3. Diagram of the stomatogastric nervous system of Procambarus clarkii (after Larimer and Kennedy 1966). Ant - anterior; Lat - lateral; N - nerve.


Ant. gastric muscle
Stomatogastric ganglion
Lateral N.
Dorsal ventricular N.
Lat. ventricular N.
Cardiac ossicle
Stomatogastric N.
(stomach)
Brain
Superior oesophageal N.
Suboesophageal ganglion
Ant. median N.
Oesophageal ganglion
Commissural ganglion
Inferior oesophageal N.

(iii) the postero-lateral nerve (posterior stomach nerve of Bullock and Horridge)which runs over the cardiac stomach on each side and branches on the pylorus, its branches extending to pyloric sensory cells on the anterior midgut.

A number of other smaller nerves run from the commissural ganglia to innervate the oesophagus and lower cardiac stomach. The posterior stomach nerves (p.s.n's of Dando 1969) are branches of the mandibular nerves from the anterior ventral ganglion and are paired sensory nerves which terminate peripherally around the insertion of the posterior gastric muscles on each side of the gastric mill.

From the oesophageal ganglion a median stomatogastric nerve (nsgs) runs dorsally to the median stomatogastric ganglion, lying between the anterior gastric muscles on the wall of the cardiac stomach, and within the anterior aorta. Posteriorly a median dorsal ventricular nerve (dvn) leaves the ganglion, soon giving rise to the paired median ventricular nerves (not shown in Fig.3) close to the ganglion and then dividing to form the paired lateral ventricular nerves (l.v.n's) which pass round the sides of the stomach. The end branches of the l.v.n's anastomose with those of the postero-lateral nerves on the pyloric stomach.

So far as is presently known (M.P. Dando, personal communication) there is only one nerve running directly from the brain to the SNS - following Orlov's (1929) terminology, this is the inferior ventricular nerve from the tritocerebrum to the oesophageal ganglion (anterior median nerve, Fig.3). The presence of a connection between the SNS and the heart (a branch of a nerve connecting the hindbrain and the stomatogastric ganglion, which reportedly runs along the aorta to the anterior heart valve) has not been confirmed in recent studies (M. Dando, personal communication).

The ganglionic neurones of the SNS are monopolar and have a variable distribution in the different ganglia. In the commissural ganglion their somata form a cortex around a central neuropile, in the oesophageal ganglion the cell bodies lie mainly anterior to the more posterior and diffuse neuropile, while in the stomatogastric ganglion there is a dorsal rind of somata over a ventral neuropile, with only very few cell bodies located ventrally.

Bullock and Horridge (1965) list the following through-fibre types in the stomatogastric ganglion (their classification being based on methylene blue studies by Orlov (1927) and the numbers refer to neurones illustrated in Figure 4:-

(i) large fibres from nsgs to bilateral stomach muscles (60)

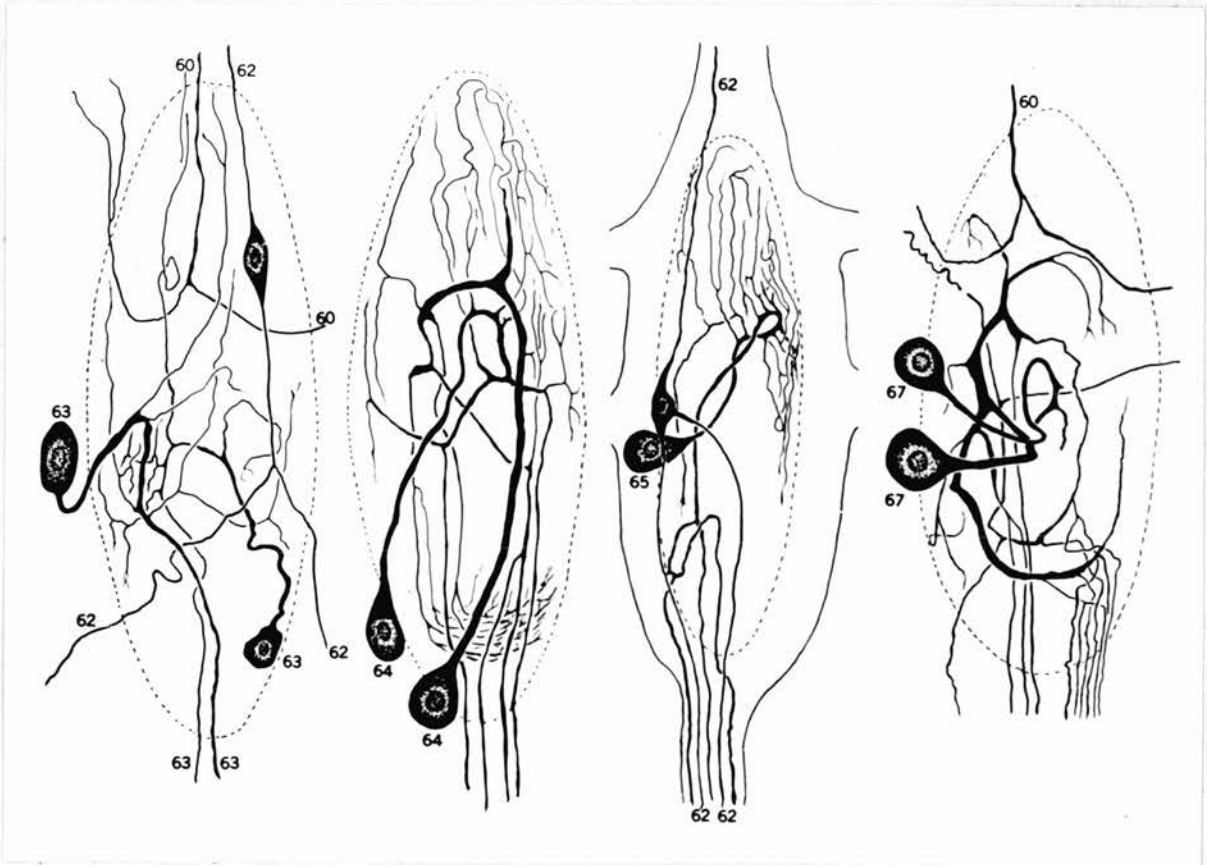
(ii) motor axons, probably with somata in the oesophageal ganglion and arborizations in the stomatogastric ganglion (61)

(iii)afferent fibres of a few bipolar or tripolar sensory cells (62) which may lie in the ganglion or on the wall of the alimentary canal (Orlov found only two or three large cells of this type).

The larger part of the stomatogastric ganglion is made up of motor neurones and their proximal arborizations. The neurones have diverse shapes and up to five main fibres (63-67, Fig.4). Orlov's methylene blue studies gave no evidence of sensory axon arborizations in the ganglion and it is considered to be a motor relay station with through-going sensory fibres (Bullock and Horridge 1965). As yet there is no physiological evidence to refute this view.

As in insects sensory cells are abundant on the foregut and are of several different types, at present being classified (D.M. Maynard and M.R. Dando, personal communication). They include cells on the cardiac

Figure 4. The gastric (stomatogastric) ganglion of the crayfish Astacus, combined from methylene blue preparations (from Bullock and Horridge 1965, after Orlov 1927). The numbered neurones are referred to in the text.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stomach whose axons run to the central nervous system in the posterior stomach nerves (p.s.n's, Dando and Laverack 1969).

The midgut in Crustacea is innervated from the posterior median nerve which runs from the posterior end of the ventral nerve cord to the hindgut (Bullock and Horridge 1965). It is possible, though not yet known, that the midgut is innervated anteriorly by the SNS. So far no sensory innervation of the midgut has been described, apart from the long processes of pyloric sensory cells whose axons run into the postero-lateral nerves.

The hindgut is innervated by posterior intestinal nerves from the last abdominal ganglion (Bullock and Horridge 1965). Branching fibres form a plexus on the hindgut, amongst which are large numbers of bipolar sensory neurones with axons to the last abdominal ganglion. Their peripheral processes penetrate the chitinous lining of the hindgut (Orlov 1925, see Bullock and Horridge 1965). Recently Winlow and Laverack (1970) have described a multiterminal anal proprioceptor in Homarus gammarus and Nephrops norvegicus, which responds to distortion of the soft cuticle bordering the anus.

(b) Physiology

Food passes from the buccal cavity, through the oesophagus to the cardiac stomach, whose anterior part is a simple sac and whose posterior part contains the gastric mill. The gastric mill is a system of articulated calcified plates, some toothed, incorporated into the wall of the stomach and moved by intrinsic and extrinsic stomach muscles. It serves to triturate food. The cardio-pyloric valve lies between the cardiac and pyloric portions of the stomach. The food-filtering apparatus is located in the pyloric stomach. The largest chewed particles are directed by a funnel to the midgut and the finest pass through a sieve into the ducts of the digestive

diverticula where enzyme secretion and absorption take place (Vonk 1960).

The myogenic or neurogenic nature of the origin of the gut rhythm is unknown and the co-ordination of the peristaltic wave has been little investigated (Bullock and Horridge 1965). From older studies, these authors concluded that there is central control of the wave initiation (and possibly its strength and velocity), nervous transmission of the wave and nervous co-ordination of circular and longitudinal muscles. They also concluded that spontaneous muscle contractions may become co-ordinated waves, that the mechanical movement may be necessary for a maintained wave, and that muscle responsiveness may be important. The SNS presumably co-ordinates stomach movements and perhaps hunger and satiation behaviour via the brain and not autonomous peripheral ganglia (Bullock and Horridge 1965).

Electrophysiological studies of the visceral nervous system are few and recent and the majority concern the SNS, particularly the stomatogastric ganglion and its associated nerves. Other studies include those on mouthpart proprioceptors (Laverack and Dando 1968; Moulins 1969), an anal proprioceptor (Winlow and Laverack 1970) and neuromuscular physiology (Maynard and Atwood 1969).

Sensory cells have been described in the p.s.n's of Homarus gammarus (Dando and Laverack 1969) which respond to normal movements of the gastric mill but not of the pyloric stomach (Fig. 5). Their dendrites are believed to end, without obvious terminal specialization, on connective tissue. Distortions of the connective tissue of the gut, caused by movement of the ossicles of the gastric mill, probably cause the generation of action potentials close to the gut. The units described were all of intermediate phaso-tonic type similar to the sensory unit of the stomatogastric ganglion studied by Larimer and Kennedy (1966, see below) in the crayfish. Repetitive

stimulation of the p.s.n. altered output in nerves from the stomatogastric ganglion (Fig.5) and in a few preparations stimulation of the central end of a cut p.s.n. evoked normal gut movements. Input from the sensory cells of the p.s.n. therefore influences the output of the ganglion and hence stomach movements.

Larimer and Kennedy (1966) described an unusual sensory cell in the crayfish stomatogastric ganglion. The cell has a bifurcating axon whose branches run in the two superior oesophageal nerves and whose terminal synapses are in the commissural ganglia. Its dendrites run, one in each of two lateral nerves from the stomatogastric ganglion (see Fig.3). In preparations with the lateral nerves connected to the stomach the cell showed constant frequency spontaneous activity which could be reset by direct stimulation of any of its four branches. Though each dendrite was autogenically active at its receptor terminal, simultaneous recordings from the two lateral nerves showed that impulses in one of them invariably preceded impulses in the other, and that the higher frequency input always determined the output. When the cardiac stomach was distorted asymmetrically, the dendrite on the side undergoing most deformation fired faster than its twin and its frequency invaded all the other branches. The unit thus produces a balanced output from an unbalanced input and probably influences the bilaterally distributed motor output to the anterior gastric muscles.

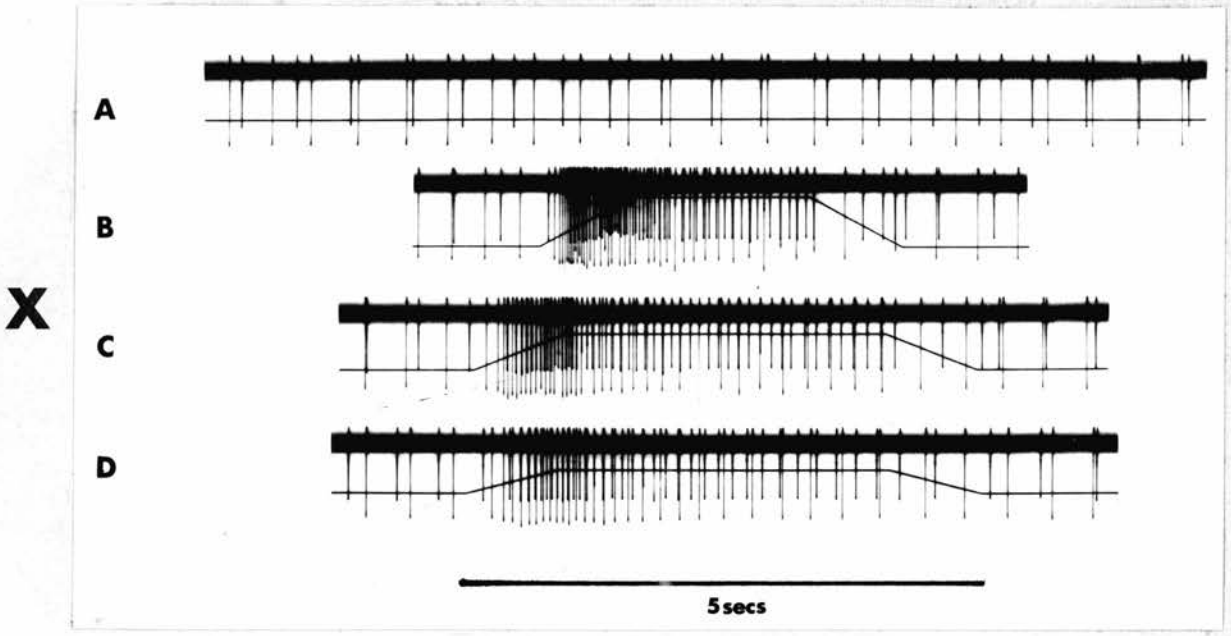
There is little available evidence for the functions of the oesophageal and commissural ganglia though it is known of the latter that they are the only places where sensory information from the gut is directed and that they produce a complex, temporally-patterned output (Dando 1969).

Studies of the stomatogastric ganglion, by contrast, have reached a sophisticated level. They have been made mainly by Maynard and co-workers

Figure 5. From Dando and Laverack (1969).
X. The response of two units in the left posterior stomach nerve of Cancer pagurus to varying sizes of movements of the central part of the pterocardiac ossicle on the same side of the mill. The movements of the gut were made by moving a probe attached to the pterocardiac ossicle. The second line on the trace shows the movement of the probe which was driven from a Servomex LF.51 Mk II waveform generator.
A. Two units firing at constant rate with no visible gut movements.
B. The response to a backward movement of 3mm of the pterocardiac ossicle in 0.9s. C. The response to a movement of 2.25 mm of the ossicle.
D. The response to a movement of 1.8 mm.

Y. Examples of the patterned output in nerves originating in the stomatogastric ganglion of Cancer pagurus. A. Output in the dorsal ventricular nerve. This record was filmed at half the film speed of record B. It demonstrates the regularity of output over a period of time. B. The top line is a record of output in the dorsal ventricular nerve. The bottom line is the output in the left outer lateral nerve of the same preparation, recorded simultaneously. The time mark applies to this trace. C. The output in the right and left lateral ventricular nerves of a preparation recorded at twice the film speed of B. Most preparations had recognizably similar units in these nerves but many were much more irregular. The cycle frequency also varied in different regular preparations.

Z. The response of two units in the left outer lateral nerve of Cancer pagurus to repetitive electrical stimulation of the cut central end of the right posterior stomach nerve. A. Normal output in the nerve, filmed at half the speed of B to E. B. Output in the peripherally isolated nerve when the small unit ceases firing. C. Two repetitive stimulations in a short series are indicated by the second line. Note inhibition and rebound. D. Record of activity after the series of stimulations. This output lasted for several minutes after which another series of stimuli were given. The 'normal' activity then returned to the nerve. E. The effect of repetitive stimulation of the posterior stomach nerve on this activity. Time mark is for B to E.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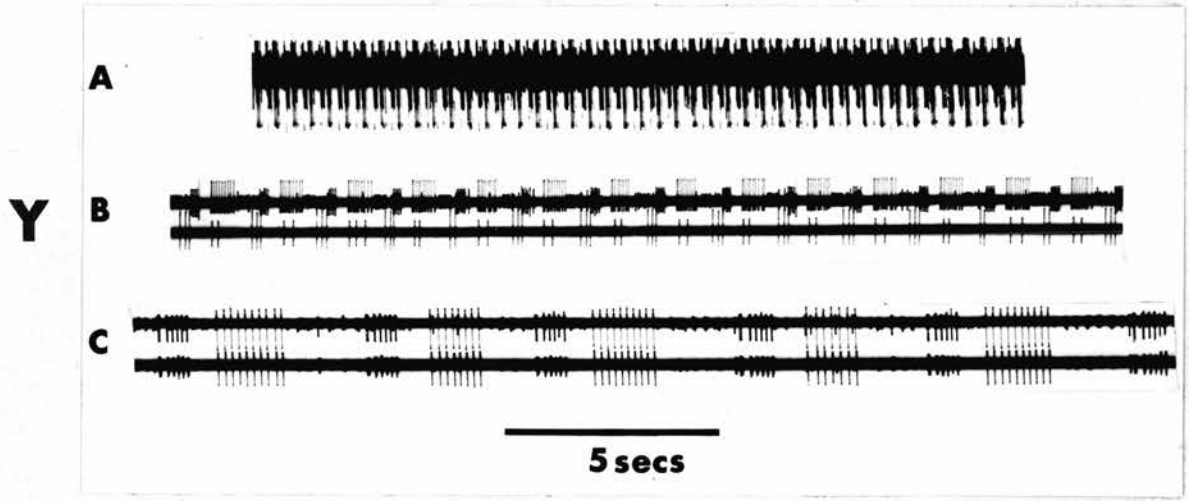
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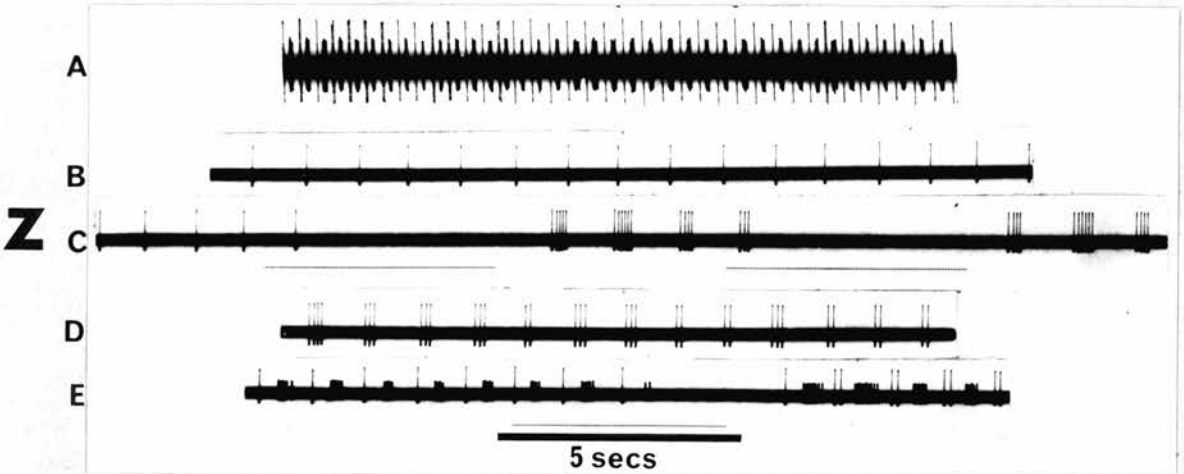
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(1962-71) on the species Scylla serrata, Homarus americanus and Panulirus argus. Maynard (unpublished) has described the ganglion of 29-35 cells as "small enough so that it is potentially feasible to obtain a knowledge of all nerve activity with current techniques, and then describe the patterns of activity and the nature of interneuron connections in terms of mechanism and the functional output of the system." Electrophysiological recording from the ganglionic neurones and associated nerves have been supplemented with light and electron microscope studies of the nervous tissue, and injection of different cell types (recognized by their electrical activity) with fluorescent dyes such as Procion yellow. The latter dye penetrates the larger cell processes well and does not kill the cells.

For the stomach muscles to act effectively they must contract in a well-ordered sequence, which inevitably requires sequential activity in the groups of stomatogastric neurones controlling the various muscles(Maynard 1966). The ganglion will produce a cyclic patterned output in response to an unpatterned input. Activity in afferent fibres from the CNS apparently triggers the ganglionic output but the neurone interaction leading to the required patterning and phase relations must occur within the ganglion itself (Maynard 1966). Of the 29 - plus neurones in the ganglion most are motor with axons running to stomach muscles and collaterals ramifying in the neuropile and synapsing with intrinsic neurones and command fibre terminals. Probably not more than three of the neurones are sensory.

In Panulirus argus, for which he has most information, Maynard (1971, in preparation) has identified 24 of the non-sensory neurones within the stomatogastric ganglion. Eleven of these are involved in control of the gastric mill and thirteen in the control of the pyloric stomach. In situ recordings from the intact animal (Morris and Maynard 1970) and in vitro

recordings from isolated ganglia and nerves (Maynard 1966, and unpublished) have shown that the pyloric and gastric mill networks have different output patterns and characteristics. Both are normally cyclic, involving sequential phase-locked activity in groups of elements but because of its regularity and ease of induction in isolated ganglia the pyloric rhythm has been most studied so far. What follows concerns the pyloric rhythm in Scylla serrata (Maynard 1969), but except for minor details, applies also to Panulirus argus.

In Scylla the pyloric neurones number 9-10. They discharge in groups segregated according to the muscles they innervate. The pacemakers of the whole pattern sequence (composed of A burst, B burst and s burst) are the four A-neurones, electrically coupled together in a positive feedback network. They discharge together in brief, high-frequency bursts of impulses and their activity feeds, probably indirectly, into the B-neurone and the 5 s-neurones, inhibiting both. The s-neurones are inhibited longer than the B-neurone which recovers and produces post-inhibitory rebound discharges. Upon s-neurone recovery, the after-discharge of the B-neurone is cut off since B is again partially inhibited. The s-neurones continue to fire until A-neurone activity recommences and the whole cycle starts again. The only feedback so far discovered to the A-neurones is the inhibitory effect of the B-neurone but it appears to be non-essential for the output sequence since it is frequently absent and even when present occurs long after cessation of A-neurone discharge. Maynard has suggested that the B-neurone may be important in other activity patterns or may prevent premature discharge of the A-cells.

In Panulirus the connections between neurones of the pyloric group have been analysed (D.M. Maynard, unpublished) and inhibitory connections of both short and long time course have been found in addition to unpolarized

electrotonic junctions. No chemically mediated excitatory synapses have been found so far among the intrinsic motor neurones of the ganglion and not all the ganglionic elements synapse with one another. All the inhibitory synapses encountered within the pyloric group are believed to be mediated by neurotransmitters and the evidence suggests (Maynard and Atwood 1969) that the inhibitory connections are monosynaptic without interposed interneurones.

Other connections within the stomatogastric ganglion include those between command interneurones and intrinsic motor neurones (Maynard, unpublished). Though not yet analysed in detail, they are known to include chemical excitatory and chemical inhibitory junctions. Connections between the elements of the gastric mill group have not yet been systematically analysed.

Since chemical synapses within the ganglion are of several different types (see above), there may be a number of different chemical mediators within the ganglion. Moreover pyloric neurones which cause excitatory depolarization in the stomach muscles they innervate, may cause inhibitory hyperpolarization in other pyloric neurones (Maynard and Atwood 1969). Either the motor neurone does not release the same chemical transmitter from all its terminals or the divergent post-synaptic effects must be explained by differing responsiveness of the post-synaptic elements.

(c) Pharmacology, endocrinology

Pharmacological studies on the crustacean alimentary canal have been generally confined to the hindgut and are few.

The spontaneous activity of the hindgut is increased by Ach and augmented by eserine; increased by eserine alone, and blocked by atropine. Contraction height is increased by NA and Factor I inhibits and blocks

the action of Ach (Florey, see Bullock and Horridge 1965). Florey inferred from these results the existence of excitatory and inhibitory innervation. Jones (1962), in agreement with Florey's work (on Procambarus, Orconectes and Pacifastacus), found that the hindgut of Astacus astacus was stimulated by Ach, A and NA. Though Florey found inhibition, Jones records that L - glutamic acid stimulated the hindgut.

There is now considerable evidence that L - glutamic acid is the excitatory transmitter at the arthropod somatic neuromuscular junction and since arthropod visceral muscle is striated, it is possible that both somatic and visceral muscles share the same excitatory transmitter (see for example Maynard and Atwood 1969; Holman and Cook 1970).

In the stomatogastric ganglion of Homarus americanus Maynard (1971b) has demonstrated the presence of a cholinesterase, primarily in glial cells enveloping neurone somata, in sheaths of identifiable large nerve fibres, and in synaptic neuropile areas. The most intense reaction was that occurring in neuropile areas. No cholinesterase activity was found in the neurone somata and the role of the cholinesterase in the neuropile is questioned. It is possible that some extrinsic neurones synapsing in the ganglion may be cholinergic. Maynard was unable to determine whether the cholinesterase in neuropile areas was associated with glial or neural elements or both.

Elofsson et al. (1968) studied the cellular localization of biogenic amines (using the Falck and Hillarp method) in the hindgut of Astacus astacus and concluded that NA and more particularly DA, occur in some nerve fibres. Osborne and Dando (1970), using the same technique, demonstrated the presence of monoamines in the stomatogastric ganglion of Homarus gammarus, in neuropile and at least some of the neurone cell

bodies. They suggested that the specific fluorescence shown by the ganglion was most probably due to DA or to a mixture of DA and NA.

Hormonal factors produced in the thoracic ganglionic mass are believed to affect the extent of permeability of the foregut of the land crab Gecarcinus lateralis to salts and water (Mantel 1968). As in insects future studies may show other gut regions and functions to be hormonally influenced.

INSECT HEART

(a) Anatomy

The abdominal portion of the dorsal vessel of insects with segmentally arranged ostia, is generally considered the heart proper (Bullock and Horridge 1965). The heart itself has a single layer of muscle cells. The segmentally arranged alary muscles insert on the wall of the dorsal vessel and on the lateral part of the dorsum on each side. The muscles, like other arthropod visceral muscles, are striated. The alary muscles, with connective tissue and pericardial cells, make up the dorsal diaphragm which separates pericardial and perivisceral sinuses. The precise role of the pericardial cells is unknown though they have been suggested to by phagocytic or to play a role in protein uptake and transport (see McCann 1970). Davey (1961a, b) believes they liberate a heart-accelerating substance when acted upon by corpora cardiaca extracts.

Heart innervation has been studied in relatively few species and appears to be variable (Bullock and Horridge 1965). The following account refers mainly to work on Orthoptera (the best-known group) and especially to cockroaches. In Periplaneta a pair of lateral cardiac cords or cardiac ganglia (Smith 1969) arise from the corpora cardiaca and run parallel along each side of the heart, fusing in the posterior part of the abdomen. 15 - 20 intrinsic neurones are distributed along each ganglion

usually situated where the segmental vessels join the heart, but occasionally where the segmental cardiac nerves join the heart (Smith 1969). Within the heart ganglion, in addition to neurones, are intrinsic cell processes and fibres of the segmental cardiac nerves from the ventral nerve cord. Short processes of the cardiac neurones run to the myocardium and alary muscles (see Davey 1964; Guthrie and Tindall 1968). In Carausius the somata of the cardiac neurones are surrounded by the arborizations of the cardiac neurone processes and of extrinsic segmental cardiac axons (Bullock and Horridge 1965). The axons of the segmental nerves run both ways along the ipsilateral cardiac ganglion (Miller and Thomson 1968). The segmental nerves also contain afferent axons from sense cells located in heart-associated connective tissue, and axons which branch in the region of the ostia run from the ventral cord in the segmental nerves. By analogy with the pericardial organs of decapods the latter may have a neurosecretory function (Bullock and Horridge 1965). Other axons from the ventral cord innervate the alary muscles.

Miller and Thomson (1968) in an ultrastructural study of cockroach cardiac innervation, found that the segmental nerves, in addition to a few ordinary glial-encapsulated axons suggested to be alary muscle motor units, contained many neurosecretory axons. They were characterized by their inclusions, containing either small electron-dense (150mμ), large electron-dense (300 mμ) or electron-opaque granules. Axons with small dense granules made neuromuscular junctions with heart muscle and were assumed to belong either to neurones in the ventral ganglia or to neurosecretory neurones of the lateral cardiac cord.

In the lateral cardiac cord itself both small and large electron-dense granule axons were found, as were axons containing large membrane-

limited 'sacs' and glial-encapsulated axons of probable motor function, both of which synapsed on the heart. The latter are believed to be axons of cardiac ganglion cells (Miller 1969). Miller also described some neurosecretory neurones in the ganglion located near the junction of segmental nerves with the lateral cardiac cord, which had electron-dense granules of varying sizes in their cytoplasm.

Some of the neurosecretory material (NSM) in the lateral cardiac cords and segmental nerves is delivered to the heart from ventral ganglia and Miller and Thomson believe it participates in a cardio-regulatory system. However, they also think that the extensive neurosecretory system of the cockroach heart may be involved in functions other than simple heart control. In addition to NSM from the ventral nerve cord and from intrinsic neurosecretory neurones, NSM is probably delivered to the heart in the nerves from the corpora cardiaca (Johnson 1966) and could derive from intrinsic cells of the corpora cardiaca themselves or from cells in the brain. The amount of such material however appears to be small, since Johnson found that the lateral cardiac nerves in the cockroach prothorax contained few axons with few neurosecretory granules when compared with the same nerves in the abdomen.

(b) Physiology

The normal insectan heartbeat is described as a forward-going peristaltic wave (see Bullock and Horridge 1965). The pumping action of the heart is attributed to the contractions of the myocardium and the alary muscles (Davey 1964) with the resultant beat depending partly on the relative timing of their respective contractions. This cannot be used as a general description however since in some insects the alary muscles are permanently contracted and in others are normally non-

contractile (see Bullock and Horridge 1965 for references).

The neurogenic or myogenic nature of the heart pacemaker is not definitely established (Bullock and Horridge 1965). McCann (1970) has commented that "neurogenicity does not necessarily derive from the fact that nerves or ganglia are present, nor does myogenicity prevail if the heart continues to beat in isolation after separation from the animal. That ganglia can be a part of the myocardium or that neural twigs persist after its excision makes true and complete denervation difficult to establish". This means that claims for myogenicity stemming from denervation experiments and histological studies where nerves cannot be demonstrated should be evaluated carefully. For example, the cockroach heart, previously classified as neurogenic on the basis of an elaborate nervous innervation and an increased heart rate and response to Ach is now indicated by some workers (see McCann 1970 for references) to be myogenic (with extensive nervous control) since beating continues after denervation; the heart maintains some activity when the animal is completely immobilized by ether vapours; the heart continues to beat after ganglionic activity is abolished by tetrodotoxin, and pacemaker-type potentials are present in myocardial cells.

Miller (1968a, b) has described the role of cardiac neurones in the cockroach heartbeat. The paired lateral cardiac cords or ganglia are electrically independent with no impulses crossing the caudal anastomosis, nervous activity apparently being co-ordinated by mechanical feedback from the moving myocardium (Miller 1968a). Spontaneously active cardiac ganglion cells are regulated specifically to fire at preferred times inducing burst groups correlated with the cardiac cycle, by movement of the heart, or can be free-running without heart movement (Miller 1968b).

Bursting occurs during the diastolic phase and neurones appear to fire when the heart diameter is increased (Smith 1969). However Miller (1969) states that burst activity in the ganglion cells does not always accompany normal heartbeat, since at times the neuronal activity is not patterned or synchronized with contractions of the heart. Spontaneously active neurosecretory cells, located in the heart ganglia near the segmental nerve junctions, are sensitive to segmental nerve stimulation but insensitive to heart movement. Miller (1968a) believes that the neurosecretory cells of the heart and the neurosecretory axons from the ventral ganglia comprise the cardio-regulatory system and he proposes the following scheme of events:-

(i) that the heart increases its beat rate when there is an increase in number of neurosecretory impulses reaching the lateral cardiac cords in the segmental nerves

(ii) that the neurosecretory impulses increase the firing rate of cardiac ganglion and cardiac neurosecretory cells

(iii) that increased activity in the cardiac ganglion cells increases their motor output to the myocardium, which causes an increase in heartbeat rate.

The rapid response of cardiac ganglion cells to segmental nerve stimulation suggested to Miller that a direct synaptic connection exists but there is the possibility that segmental neurosecretory axons increase heartbeat rate by directly synapsing with the myocardium.

In contrast to the suggestion (iii) above, Smith (1969) found that increased firing rate in neurones of the cardiac ganglion is apparently <u>not</u> required for cardio-acceleration responses for when action potentials of cardiac neurones in isolated pieces of ganglion were abolished by

tetrodotoxin inhibition, cardio-acceleration still occurred after subsequent application of corpora cardiaca extracts. He suggested that the pericardial cells may respond in such cases but did not rule out possible action of corpora cardiaca factors at nerve endings or direct effects on the myocardium.

The presence of inhibitory nerves to the insect myocardium has not been confirmed (Miller 1969; McCann 1970).

(c) Pharmacology, endocrinology

Only the action on the heart of known or postulated transmitter substances will be considered here. What follows refers to one of the best-known species, the cockroach Periplaneta americana.

Partially isolated cockroach hearts are sensitive to Ach. Concentrations up to 10^{-5}M cause an increased frequency of contraction, the effect being antagonized by curare and atropine (see Davey 1964). Davey suggested that the primary site of action of Ach might be the myocardium rather than the ganglion cells. However, Miller and Metcalf (1968) found that denervation of Periplaneta heart led to a simple myogenic beat unresponsive to atropine and Ach in concentrations up to 10^{-3}M and proposed that these compounds acted instead on the cardiac nervous system.

Specifically, Miller (1968b) found that Ach chloride increased the firing rate of isolated cardiac ganglion cells at less than 10^{-9}M concentration, and that the effect was blocked by D-tubocurarine chloride and atropine sulphate. Stimulus-response and drug studies did not reveal the presence of a cholinergic synapse in the cockroach cardiac nervous system and Miller therefore suggested that Ach and cholinomimetics act on the cardiac ganglion cells at an unspecified cholinergic-sensitive site rather than at a synapse membrane. He noted that a difficulty to studying in detail the response of the cardiac nervous system to

cholinergic compounds was the lack of a clear-cut duplication of the cholinergic effects by simple stimulation. The results of stimulation of both segmental and lateral cardiac nerves were not affected by the presence of cholinolytic compounds, nor did these compounds greatly alter heart activity or spontaneous ganglion cell nervous activity.

Adrenaline and other amines with a phenolic nucleus like tyramine and dopamine excite the isolated heart of Periplaneta (see Davey 1964). Miller and Metcalf (1968) found that when the denervated heart was perfused with A, NA, DA, tryptamine or 5-HT, it responded with an increased rhythmic contraction rate. These authors believe such compounds act on the myocardium and not on the cardiac nervous system though Miller (1968b) did find that 50 µl of 10^{-6}M DA caused a rapid increase in rate of ganglion cell impulse production as recorded from the isolated cardiac cord. The acceleratory action of 5-HT far exceeded that of Ach, A or NA (see Treherne 1966).

L - glutamate and GABA at 10^{-3}M, when dropped onto the isolated cockroach heart had no definite effect on the recorded myocardiogram and therefore no effect at the cardiac neuromuscular junction (Miller and Metcalf 1968).

Maynard (1960) has indicated the difficulty of interpreting pharmacological studies on the arthropod heart since changes in rate or amplitude of contraction do not discriminate between several possible sites of drug action (neurones, intra-ganglionic synapses, heart neuromuscular junctions or heart muscle). Further, Davey (1964) suggested that substances affecting the heart may affect myocardium and alary muscles differentially.

While spontaneous ganglion cell activity is very sensitive to

cholinergic compounds there is no evidence for a cholinergic synapse in the cardiac nervous system (Miller 1968a). There is no information on the chemical transmitter at any one of the many possible neuro-neuronal or neuromuscular synapses of the heart though Johnson (1966) has suggested the possibility that material present in neurosecretory axons synapsing with heart muscle may be a catecholamine (CA).

Several factors have been extracted from the corpora cardiaca and CNS of insects which affect the beating of the isolated heart (see for example Natalizi et al. 1970) but Davey (1964) believes there is little positive evidence of a role for them in the normal functioning of the heart. The factors appear to be mainly peptides and though 5-HT has been found in the corpora cardiaca it is believed to contribute little to the activity of extracts (Natalizi and Frontali 1966). Using the Falck and Hillarp method, Frontali (see Natalizi and Frontali 1966) failed to obtain evidence for the presence of CA's in cockroach corpora cardiaca.

Davey (1961a, b) reported that a peptidic heart-accelerating substance from the corpora cardiaca indirectly affects the heart by acting on the pericardial cells, causing them to release a pharmacologically active substance. While the pericardial cells do have secretory characteristics it is believed (Kater 1968, see McCann 1970) that the cardioaccelerator from the corpora cardiaca acts directly on the heart. Smith (1969) found that corpora cardiaca extracts cause isolated cardiac ganglion neurones to increase their spontaneous firing rate. The cardiac ganglia are therefore possible sites of action for cardio-accelerators.

CRUSTACEAN HEART

(a) Anatomy

The primitive crustacean heart is a tube of striated muscle running the length of the body within a blood-filled sinus, the pericardium. Blood returning from the gills enters the pericardial cavity by way of the branchio-pericardial veins and then passes into the heart through the ostia, a pair of valved openings in each segment. In decapods the heart essentially occupies only three segments (Carlisle 1964). The heart is suspended in the pericardium, whose roof and lateral walls are composed of subcutaneous connective tissue of the outer body wall, extensions of the pericardial septum, and often the longitudinal somatic musculature of thorax and abdomen. The floor and anterior wall of the pericardium are formed by the elastic pericardial septum (connective tissue) and in some groups including the Malacostraca, the alary muscles are associated with this septum (Maynard 1960). The alary muscles insert on the lateral body wall and the dorsal septal surface.

The decapod crustacean heart possesses an intrinsic ganglion generally of nine cells (16 in Astacus), situated on its inner dorsal surface. The cell axons form a local system and are not known to extend beyond the heart boundaries (Bullock and Horridge 1965). The small and large (mostly multipolar) intrinsic neurones, their branched axons, axons of the dorsal nerves, and neuropile constitute the cardiac ganglion. The large, unlike the small cell bodies are surrounded by a network of axon arborizations from regulator fibres of the dorsal nerves (see below). In the neuropile branching processes of intrinsic and regulatory fibres make contacts.

Three pairs of extrinsic regulatory axons (two excitatory and one inhibitory) run from the ventral nerve cord to the heart. At first they

run in separate regulator nerves (of which most fibres are neurosecretory to the pericardial organs), then they pass through the lateral pericardial plexus (see Bullock and Horridge 1965 Volume II, Figure 17.13) and on each side two excitatory, one inhibitory and a number of smaller fibres collect into a dorsal nerve which enters the heart. The regulator fibres arborize in the ganglion, running to each cardiac neurone and sending collaterals to heart muscle (Horridge 1968). In Astacus and Squilla the smaller fibres of the dorsal nerve run directly to heart muscle with no connections in the ganglion (see Bullock and Horridge 1965).

Other nerves supplying the heart are the paired segmental pericardial nerves from the thoracic cord to the muscles of the pericardium, the heart valves and the pericardial organs (P.O's) and the reported anterior median aorta nerve from the SNS to the anterior heart valve. No terminal connections have been found between the fibres of the segmental nerves and those of the ganglionic neurones, though the two sets of fibres are in close proximity at the heart valves (Bullock and Horridge 1965).

The neurohaemal pericardial organs are plexuses or nerve trunks spanning the openings of the branchio-pericardial veins into the pericardium (see Maynard and Welsh 1959). The trunks have a central core of nerve fibres, connective tissue and blood vessels, and a cortex of neurosecretory terminals (Maynard and Maynard 1962). The terminals are separated from the haemolymph by a thin acellular epineurium. Many of the secretory fibres of the P.O's originate from cell groups in the ventral thoracic ganglion. Also contributing to the organs' structure however, are cells with somata located within the P.O's, some of which show secretory characteristics (Belamarich and Terwilliger 1966). The lateral pericardial plexus forms a major part of the P.O's.

The P.O's contain elementary neurosecretory granules (Knowles 1962; Maynard and Maynard 1962). In Squilla mantis Knowles distinguished two fibre types with the electron microscope, type A containing electron-dense spherical or ovoid vesicles about 1500 Å in diameter resembling neurosecretory inclusions and type B containing more irregularly-shaped vesicles about 1200 Å in diameter accompanied by 500 Å diameter vesicles. The large vesicles in A and B fibres were membrane-limited but in type B fibres membrane and dense core were separated. Knowles (1964) believes the dense-cored vesicles of type B fibres resemble those thought to contain 5-HT or NA. Evidence from selective staining led Maynard and Maynard (1962) to suggest that there may be three kinds of secretory terminal in the P.O's of Carcinus maenas. Two populations of electron-dense, membrane-limited granules (1700 and 1400 Å in diameter) were found in separate terminals. A third type of terminal contained vesicles about 300-500 Å in diameter. Differing population types were found in Cancer irroratus and Libinia emarginata (Maynard and Maynard 1962) and in Cancer borealis (Terwilliger et al. 1970).

(b) Physiology

Distension of the heart at diastole is produced by the pull of the elastic supporting ligaments and relaxation of the myocardium (Taylor 1970). At systole blood is forced anteriorly.

A regulated neurogenic heart is the rule in arthropods (Bullock and Horridge 1965) and in decapods the cardiac ganglion is believed to be the pacemaker. Ganglionic activity precedes electrical and mechanical responses of the myocardium. In decapods the striated heart muscle contracts in response to regular periodic bursts of nerve impulses from the cardiac ganglion (Cooke 1966; Anderson and Cooke 1969) and will only contract in the presence of one or more ganglion cells. A normal burst of activity

in the ganglion starts among the four smaller posterior cells and spreads to the five larger anterior neurones (Maynard 1966). Though the two groups of neurones are normally regarded as pacemaker and follower cells respectively (see Maynard 1960; Bullock and Horridge 1965) Maynard believes it may be misleading to classify them thus, since spontaneity is not the prerogative of one cell type and parts of the isolated ganglion may continue to show burst activity in the absence of small cells (see also Connor 1969). Though the full details of burst initiation and intercellular co-ordination are not yet completely understood, nevertheless it is known that there are both normal polarized synaptic and unpolarized electrotonic interactions of importance between neurones (Maynard 1966).

In the Decapoda the frequency of the heartbeat is controlled by the acceleratory and inhibitory fibres from the CNS (see Taylor 1970) which affect the ganglionic neurones at many physiologically diverse loci (Bullock and Horridge 1965). Rhythmical activity has been recorded from the accelerator nerves of Astacus pallipes (Taylor 1970) but it depends upon the integrity of the accelerator nerve. Taylor believes this indicates that feedback from the heart triggers or clamps central nervous activity and that sensory inflow is along the nerve itself. Activity in the sectioned nerve was of a less patterned type. Taylor suggested that the very high levels of spontaneous activity recordable in the cardioregulator nerves in his experiments may indicate that they have some form of tonic control over heart rate in the intact animal. The bursting unit he recorded may have been either the accelerator fibre proper or else a fibre supplying the P.O's and causing release of cardio-acceleratory hormone. Electrical stimulation of the cardio-acclerator nerves of Libinia emarginata reportedly caused release of excitatory material from the P.O's (Cooke 1964).

Inflation of the isolated or in situ Malacostracan heart increases its beat amplitude and frequency. Stretch is apparently the effective stimulus (Maynard 1960) and causes increased discharge in cardiac neurones. The anatomy of the dendritic arborizations of the neurones indicates that they may be the receptive elements in which case both sensory and motor elements of the intrinsic reflex would be present in the same cell. This reflex would provide a direct feedback to the ganglion (in addition to the feedback via the cardio-accelerator nerve described above) and in the normally beating heart probably tends to synchronize the spontaneous ganglion discharge with a distended and filled myocardium (Maynard 1960).

Cooke (1966) has summarized the functioning of the cardiac ganglion in the following way: - "The ganglion integrates the spontaneity of its own cells, the effects of a pair of inhibitor and two pairs of accelerator fibres from the CNS and the effect of muscle stretch on dendritic processes from the ganglion cells to produce rhythmic bursts of impulses in efferent axons to the heart muscle. These bursts vary in their frequency, duration and the number and frequency of units comprising each burst in response to physiological demands."

(c) Pharmacology, endocrinology

Low concentrations of Ach and analogues (down to 10^{-9} gm/ml) tend to stimulate the hearts of Malacostracans while high concentrations inhibit (Maynard 1960). For many years the heart accelerator axons were thought to be cholinergic but this is no longer believed (see Bullock and Horridge 1965; Treherne 1966). While crustacean tissue contains Ach and acetylcholinesterase, the cardiac ganglion and accelerator fibres contain no detectable amounts of Ach (see Treherne 1966). Maynard (1971b) detected only very small amounts of cholinesterase in the ganglion and cholinesterase

activity was found primarily in glial cells ensheathing large and small neurone somata and their processes within the ganglion. There was only an extremely faint reaction in neuronal cytoplasm, and the neuropile had no intensely cholinesterase-positive areas. Maynard (1971b) points out that though lack of cholinesterase activity may indicate the absence of cholinergic neurones or their processes, it does not mean that the neurones being considered are non-cholinoceptive. She suggests that much of the cholinesterase in glial elements in crustaceans may be protecting non-synaptic cholinoceptive sites from small fluctuations in the level of cholinergic compounds in the milieu and that in the cardiac ganglion where little or no 'protective' cholinesterase is present it may be important for its functioning that the neuronal membranes are readily accessible to any circulating cholinergic material.

A, NA, DA, 5-HT, 6-hydroxytryptamine, 5, 6-dihydroxytryptamine, glutamic acid andsome other amino acids excite the decapod crustacean heart (Cooke 1966) with the threshold of response being ten-fold lower for 5-HT than for the other compounds.

CA's have been found only in very low concentrations in crustaceans (see Treherne 1966) and not so far in the heart. 5-HT has been extracted from the P.Os of decapods (Maynard and Welsh 1959; Carlisle 1964; Belamarich and Terwilliger 1966). 6-hydroxytryptamine and 5, 6-dihydroxy-tryptamine also reportedly occur in the P.O's but Belamarich and Terwilliger found only 5-HT in the P.O's of Cancer borealis and think the simultaneous existence of the other two compounds is uncertain.

A recent paper (Cooke and Goldstone 1970) describes the localization of a CA and 5-HT in crab P.O's by means of the histochemical fluorescence method. Both green- and yellow-fluorescing axons (containing

a CA and 5-HT respectively) innervate the P.O's in addition to neurosecretory axons. All the fibres enter the organs by way of segmental nerves from the ventral ganglion. One large green-fluorescing axon described arises from a green fluorescent cell in the commissural ganglion, and travels in the commissure almost to the brain where it apparently closely approaches another large green fluorescent cell. The axon then doubles back on itself and passes via the ventral ganglion to the P.O's. Cooke and Goldstone believe that cell bodies in the commissural ganglia may be the source of all the green-fluorescing axons and terminals in the P.O's. They were unable to locate the cell bodies of the 5-HT - containing fibres. Data to be presented in a later paper (see Cooke and Goldstone 1970) establishes that the green fluorescence represents the intracellular localization of DA, and the yellow fluorescence that of 5-HT.

5-HT increases the rate and amplitude of beating of the isolated heart of Homarus americanus when introduced in the internal perfusion fluid, in a similar way to extracts of the P.O's of Cancer borealis (Cooke 1966). Maynard and Welsh (1959) believed that the amount of 5-HT in the P.O's could not account for the intense physiological action of extracts on the decapod crustacean heart. Hearts made unresponsive to 5-HT still responded to P.O extracts (Cooke 1962). Extracts of the P.O's contain, in addition to 5-HT, a cardio-acceleratory polypeptide (see Terwilliger et al. 1970 for references). Terwilliger and co-authors have shown that this peptide, in Cancer borealis, is probably associated with membrane-limited electron-dense granules (ca. 1500 Å in diameter) seen in electron micrographs of the P.O's. 5-HT appears to be associated with a different particle and so far its possible role in the regulation of the crab heartbeat remains unknown.

Berlind et al. (1970) have attempted to demonstrate a role for the monoamine-containing terminals of the P.O's in peptide neurosecretion but concluded as a result of their experiments, that peptide neurosecretory release is controlled by the electrical activity of the neurosecretory cells and is not detectably influenced by the DA- or 5-HT - containing terminals. However, they did not rule out the possibility of long term interactions between neurosecretory and monoamine-containing elements. For the moment, though, the role of the latter elements is unknown.

GABA decreases the duration and frequency of bursts in the cardiac ganglion. The heart inhibitory transmitter is not known to differ from that in inhibitory motor axons to somatic muscles (Bullock and Horridge 1965).

The neurohaemal P.O's of the spider crab Libinia emarginata have been shown to release a neurosecretory hormone (peptide) upon electrical stimulation of the cardioaccelerator nerves (Cooke 1964; Berlind and Cooke 1970; Berlind et al. 1970). The organs, while ideally situated for the release of cardio-acceleratory agents in the path of blood returning to the heart from the gills, are also well-sited to release active substances which may be carried in the blood to target organs elsewhere in the body (Maynard and Welsh 1959; Belamarich and Terwilliger 1966). Such active substances could include the DA and 5-HT of the monoamine-containing terminals. Maynard and Welsh (1959) have suggested the possible analogy of the P.O's with the vertebrate adrenal medulla, affecting the general activity state of the animal.

Table 1. Neurotransmitter and neurosecretory influences on arthropod viscera.*

ARTHROPOD GUT	Occurrence of active substances	Effects of active substances
Insects	L-glutamic acid, L-aspartic acid and a hormone in the gut and its nervous system.	Ach, 5-HT, tryptamine, 5,6-dihydroxytryptamine, DA, NA, L-glutamic and L-aspartic acids reportedly have an excitatory effect.
	An indolalkylamine (?) in argentaffin cells of the colon.	Ach and DA are reported by some authors to have an inhibitory effect. Extracts of corpora cardiaca, corpora allata, brain and suboesophageal ganglion have an excitatory effect.
Crustaceans	NA and DA in some nerve fibres of the hindgut. DA in some cells of the commissural ganglia. DA and/or NA in neuropile and some neurone somata of the stomatogastric ganglion.	Ach, A, NA, Factor I and L-glutamic acid have an excitatory effect on the hindgut. An inhibitory effect of L-glutamic acid is also reported.

ARTHROPOD HEART	Occurrence of active substances	Effects of active substances
Insects	A pharmacologically active substance from the pericardial cells?	Ach, 5-HT, tryptamine, A, DA and NA have an excitatory effect. Ach is believed to act on the cardiac nervous system and the other compounds on the myocardium. DA however may also affect the ganglion cells. Tyramine has an excitatory effect. Extracts of corpora cardiaca and CNS have an excitatory effect.
Crustaceans	5-HT, DA and a cardioacceleratory peptide (s) in the pericardial organs. 6-hydroxytryptamine, 5, 6-dihydroxytryptamine also reportedly occur.	A, NA, DA, 5-HT, 6-hydroxytryptamine and 5, 6-dihydroxytryptamine have an excitatory effect. Ach - low concentrations are excitatory, high concentrations inhibit. GABA has an inhibitory effect on the cardiac ganglion. The pericardial organs contain cardioacceleratory peptides.

* The table summarizes information from the introduction (where the appropriate references may be found) and refers to several different species.

II. MATERIALS AND METHODS

Specimens of *S. gregaria* and *L. m. migratorioides* were obtained from breeding stocks maintained at the Gatty Marine Laboratory and the Department of Zoology, University of Dijon, respectively. Specimens of *H. gammarus* were obtained locally (St. Andrew's, Fife) and kept in tanks of circulating sea water.

1. Anatomy-methylene blue staining.

Locusts

Legs and wings of mature adults were removed and the thoracic cavities were injected anteriorly and posteriorly with a total of 0.5 ml. of leuco-methylene blue solution (diluted with 0.75% solution of sodium chloride). Intra-vitam staining was continued for two hours. After removing one side wall of the thorax the whole bodies were immersed in cold (4°c) 10% ammonium molybdate solution (three changes in the first thirty minutes) and fixed for twenty-four hours. They were then washed for two hours in distilled water and stained nerves were traced by removing obscuring structures such as Malpighian tubules. Permanent preparations of various regions of the gut wall were made by rapidly dehydrating tissue in absolute alcohol, removing the cuticular gut lining where necessary, clearing the tissue in Xylol and mounting in Xylol dammar.

Homarus

Nerves and ganglia were either stained *in situ* by adding a few drops of methylene blue solution to the dissecting dish or by isolation and immersion in a very dilute solution of methylene blue in sea water, left overnight at 4°c in a covered watchglass. The staining of the ganglion and nerves was for personal reference only since the anatomy of the system has previously been described (see Bullock and Horridge 1965).

2. Histology - light and electron microscopy

Fixation and embedding - locusts

Mature adults were used (mainly males for ease of dissection). The ingluvial ganglion and nerves of S. gregaria were fixed either in

a) cold (4°c) 1% osmium tetroxide in 0.75% sodium chloride solution for 30 minutes or in

b) cold (4°c) buffered 2% glutaraldehyde overnight, followed by a buffer rinse and post-fixation in 1% buffered osmium tetroxide solution for 1 hour. Dehydration was done in acetone and embedding in Araldite.

The ingluvial ganglion and nerves of L.m. migratorioides were fixed as detailed in b) above, dehydrated in an alcohol series and embedded in Epon/Araldite.

Fixation and embedding - Homarus

The stomatogastric ganglion and adjacent lengths of the stomatogastric and dorsal ventricular nerves were fixed in one of the following ways:-

a) in a cold (4°c) 1% solution of osmium tetroxide in sea water for 30 minutes

b) in a cold (4°c) 1% buffered solution of osmium tetroxide for 1 hour

c) in a cold (4°c) buffered 2% glutaraldehyde solution overnight, followed by a cold buffer rinse and post-fixation for 1 hour in 1% buffered osmium tetroxide solution.

In all cases dehydration was done in acetone and embedding in Araldite.

Fixation of the ganglion appeared to be less affected by the different procedures used than fixation of the nerves, where osmium tetroxide alone resulted in round cross-sections to the fibres but more membrane damage than double fixation. Fixation of more material would reveal whether these were true or chance differences.

Staining

Sections for light microscopy were cut on Reichert or L K B microtomes and stained with a dilute solution of toluidine blue. Thin sections for electron microscopy were cut on the same microtomes, mounted on coated or uncoated copper grids, double-stained with lead citrate and uranyl acetate solutions, and examined with Hitachi HS 75 or AEI EM6B electron microscopes.

3. Histochemistry - Falck and Hillarp technique

S. gregaria

Ingluvial ganglia from adults of both sexes were dissected out in cold locust ringer (Hoyle 1953) and transferred on small squares of ringer-moistened filter paper to propane cooled with liquid nitrogen. Tissue was freeze-dried at -40°c and 10^{-3} TORR for at least twenty-four hours and up to three days, then exposed at 80°c to formaldehyde vapour for one hour (R.H. of paraformaldehyde 70%), and finally was infiltrated under reduced pressure with paraffin wax. Sections (mainly transverse) were cut of the ganglia, 8-10 µm thick, were mounted using thin glass slides and coverslips in liquid paraffin, and examined under dark-field illumination with a Leitz U.V. fluorescence microscope, using appropriate filters. For control ganglia, exposure to formaldehyde vapour was the only step omitted from the above procedure.

Some sections from formaldehyde-treated ganglia were immersed in alcoholic sodium borohydride solution and re-examined for specific fluorescence. Other sections were observed during prolonged exposure (up to two hours) to U.V. irradiation.

III. RESULTS

1. Anatomy - methylene blue staining

Locusts

Methylene blue staining of the ingluvial ganglion and main associated nerves of L.m. migratorioides showed essentially the same plan as in S. gregaria, with the various nerves innervating similar regions of the gut. Occasionally ncl and nlg arise separately from the ganglion and not as a common trunk.

The ganglion itself is similar to that of S. gregaria. Sensory cells have the same distribution on the gut as in S. gregaria. Figure 6 shows a sensory cell from the foregut of S. gregaria. This cell is multipolar.

Homarus

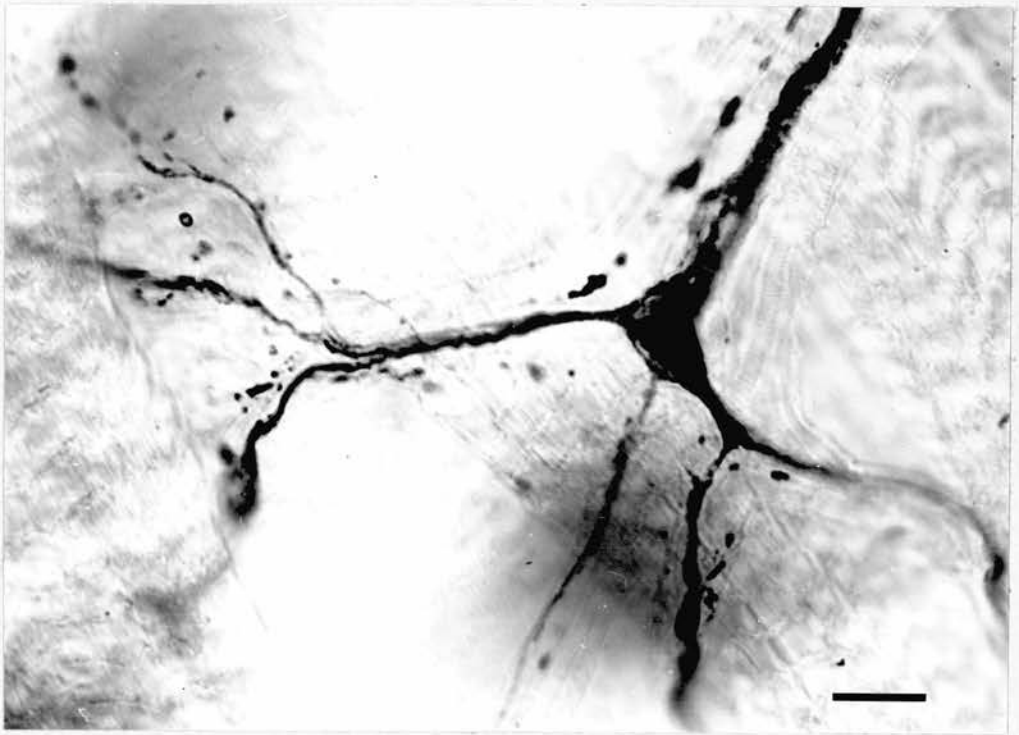
In stomatogastric ganglia stained with methylene blue up to 29 cell bodies were counted. The true count is probably slightly higher than this since some overlapping of somata occurs, making accuracy difficult to achieve; all somata may not take up stain equally in one preparation; and occasional sensory cells of the ganglion are sometimes located within the ganglion, sometimes outside it (Orlov 1926a). The count of cell bodies compares with 30-35 in Scylla serrata (Maynard 1969) and 28-31 in Panulirus argus (D.M. Maynard, unpublished observations).

2. Histology - fine structure

Locusts - ingluvial ganglion

The ingluvial ganglion has the typical invertebrate ganglion structure of a central neuropile surrounded by a cortex of neurone somata and glial cells. Outside the cortex is a definite layer of glial cells, the perineurium, enclosing which is an acellular fibrous sheath or neural lamella (continuous with the nerve sheaths). In toluidine blue-stained

Figure 6. Photomicrograph of a multipolar sensory neurone on the foregut of Schistocerca gregaria, stained with methylene blue. Scale mark 20 µm.



sections some of the neurone somata appeared grouped (Fig. 7A). This might indicate a common function for the cells in the group e.g. innervating the same muscle or gut region, or different muscles with similar function. It is also apparent from Figure 7 that there are regions, apart from nerve exits, where there are no cortical neurones between the neuropile and the neural lamella (see also Fig. 9A).

Glial cells

There are three main types of glial cell in the insect ganglion (Wigglesworth 1960).

a) Perineural glial cells form a layer outside the cortex. They are flattened cells with elongate nuclei (Fig. 9A) and many mitochondria.

b) Cortical glial cells have processes which closely invest the neurone somata and sometimes indent the neuronal cytoplasm (Fig. 8).

c) The glial cells adjacent to the neuropile (Figs. 7B, 8) have processes which penetrate the neuropile to invest many of the fibres there, separating them from one another (Figs. 11, 13A). Such glial insulation is absent between many fibres (Fig. 13B).

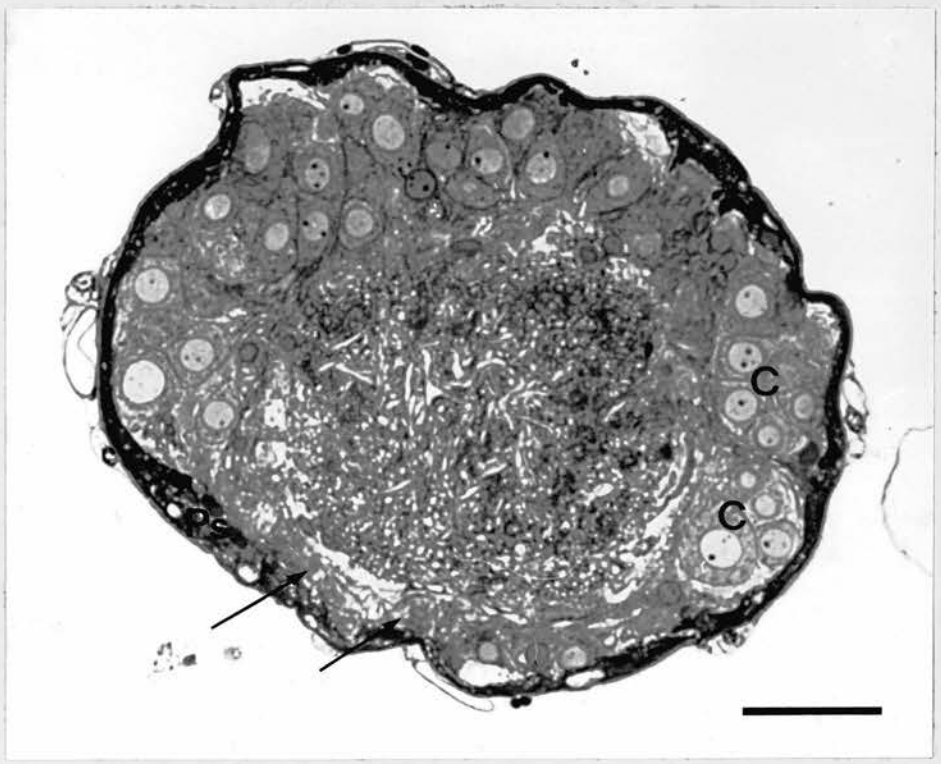
Type b) and c) glial cells frequently have round nuclei, averaging 9 μm in diameter, but they may sometimes be oval in section. There may be some overlap of function between the two types.

Neurones

Since the somata of invertebrate sensory neurones are generally located peripherally (Bullock and Horridge 1965) the neurones of the ingluvial ganglion are assumed to be motor and/or interneurones. However a small number of sensory cells may be located near or within the decapod crustacean stomatogastric ganglion (Orlov 1926a; Larimer and Kennedy 1966;

Figure 7. Photomicrographs of a longitudinal section through the ingluvial ganglion of Schistocerca gregaria, stained with toluidine blue. A. In one region of the section (arrows) there are no neurone somata between the neuropile and the sheath. Some of the neurone cell bodies appear to be grouped (C). Scale mark 50 µm. B. The neuronal nuclei are round or oval in section and often show prominent nucleoli. The nuclei of the glial cells are smaller and more densely stained than the neuronal nuclei. The glial cells adjacent to the neuropile probably send processes into the neuropile. Scale mark 10 µm. gn - glial cell nucleus; n - nucleus; N - neurone soma; np - neuropile; Ps - perineurium and sheath.

A



B

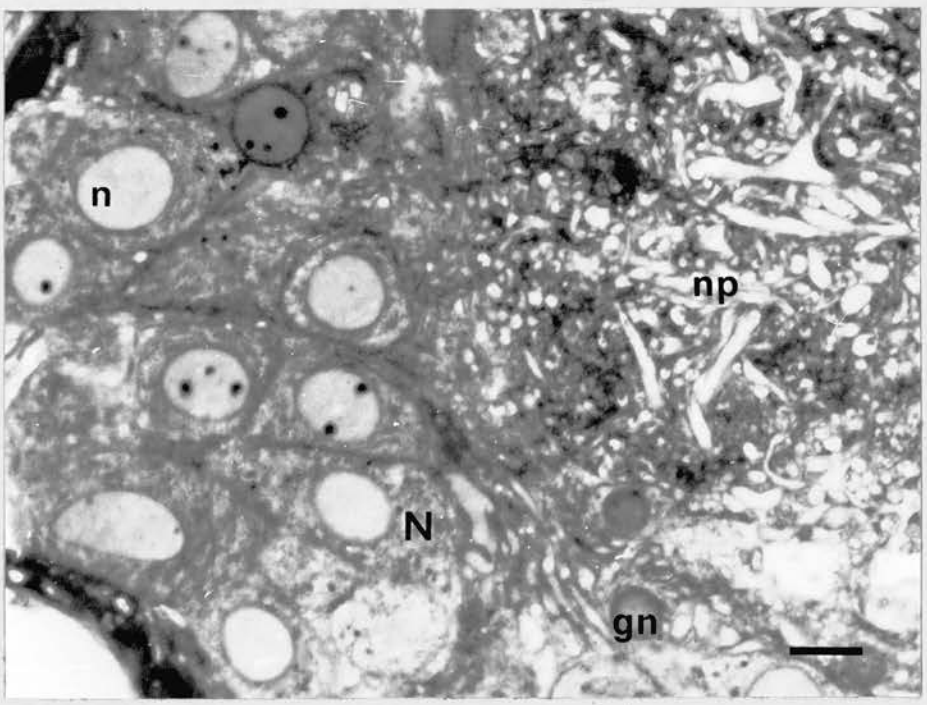
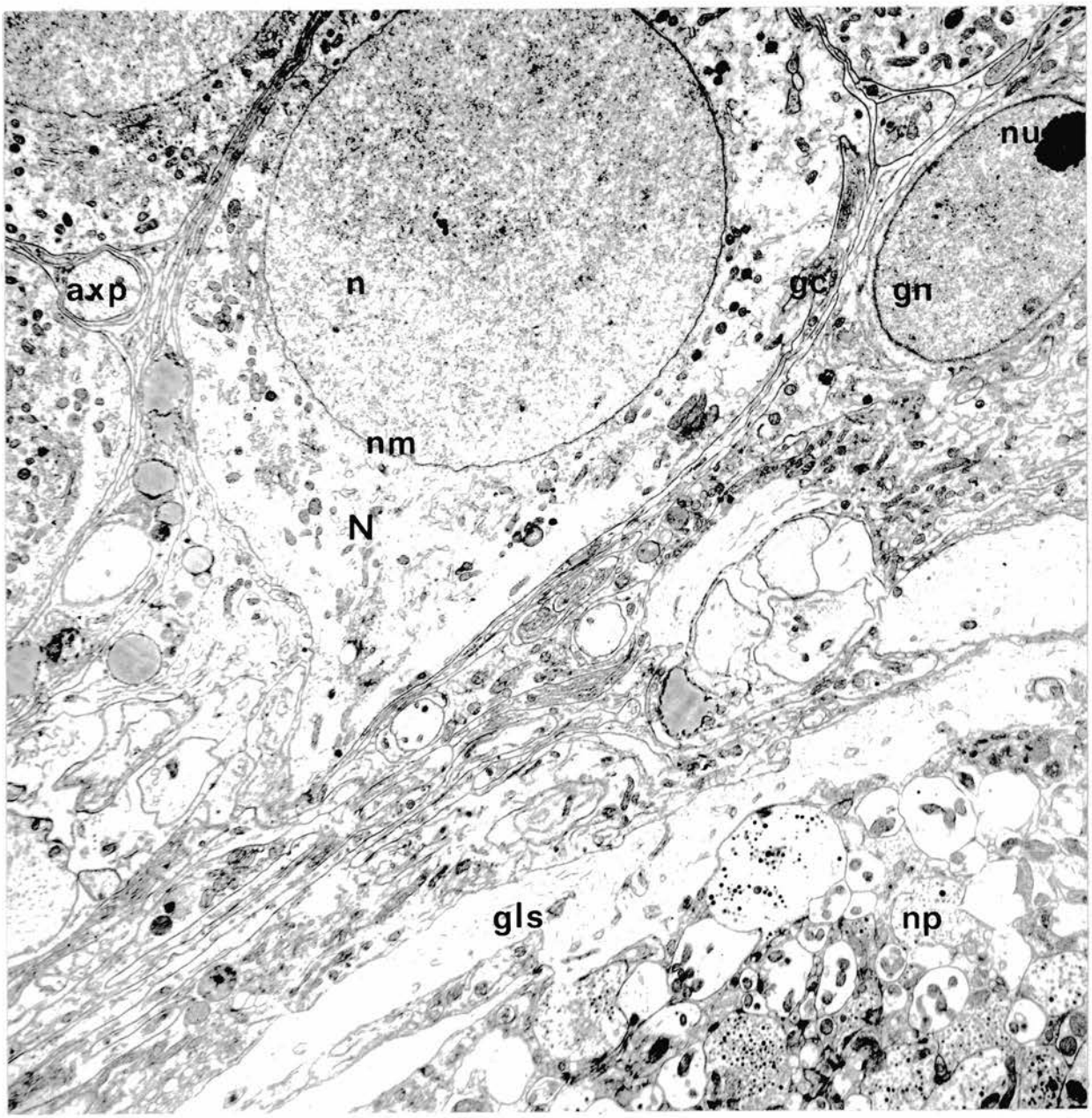


Figure 8. Electron micrograph of the ingluvial ganglion of Schistocerca gregaria showing parts of the cortex, glial lacunar system and neuropile. The glial cell at the right of the field may be one which contributes processes to the glial lacunar system. It may also send cytoplasmic extensions into the neuropile. Nerve fibres seen in transverse section in the glial lacunar system may be directed towards the neuropile, as may those near neuronal cell bodies. In the neuropile several fibre sections may be seen which contain densely stained vesicles. axp - axon process; gc - glial cytoplasm; gls - glial lacunar system; gn - glial cell nucleus; n - nucleus; N - neurone soma; nm - nuclear membrane; np - neuropile; nu - nucleolus. Magnification x 7200.


axp
n
nm
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gc
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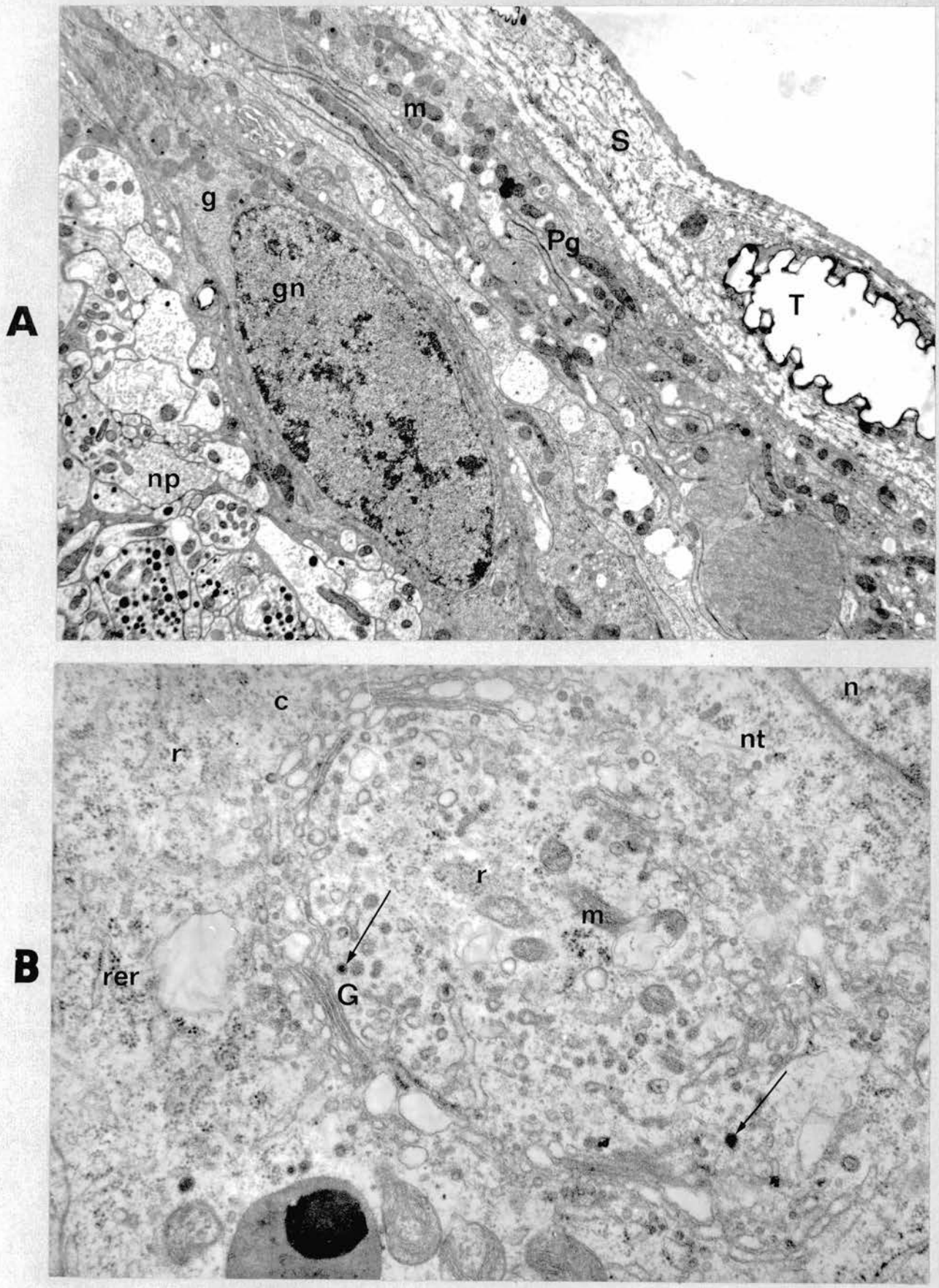
Figure 9.A.Electron micrograph of the ingluvial ganglion of Locusta migratoria showing a region where neuropile and perineurium are not separated by the neuronal cortex. Note the flattened perineural cells with numerous mitochondria. In this figure the glial lacunar system is not evident. Magnification x 7500.

B.Electron micrograph of the ingluvial ganglion of Locusta migratoria showing part of a neurone with a Golgi body. Densely staining material may be seen within some channels of the Golgi body and a few dense-cored vesicles are present (arrows). Magnification x 25,300.
c - cytoplasm; g - glial cell; G - Golgi body; gn - glial cell nucleus; m - mitochondrion; n - nucleus; np - neuropile; nt - neurotubule; Pg - perineural glial cell; r - ribosomes; rer - rough endoplasmic reticulum; S - sheath or neural lamella; T - trachea.


A
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D.M. Maynard, unpublished observations) and it is therefore possible that the same situation could prevail in insect visceral ganglia.

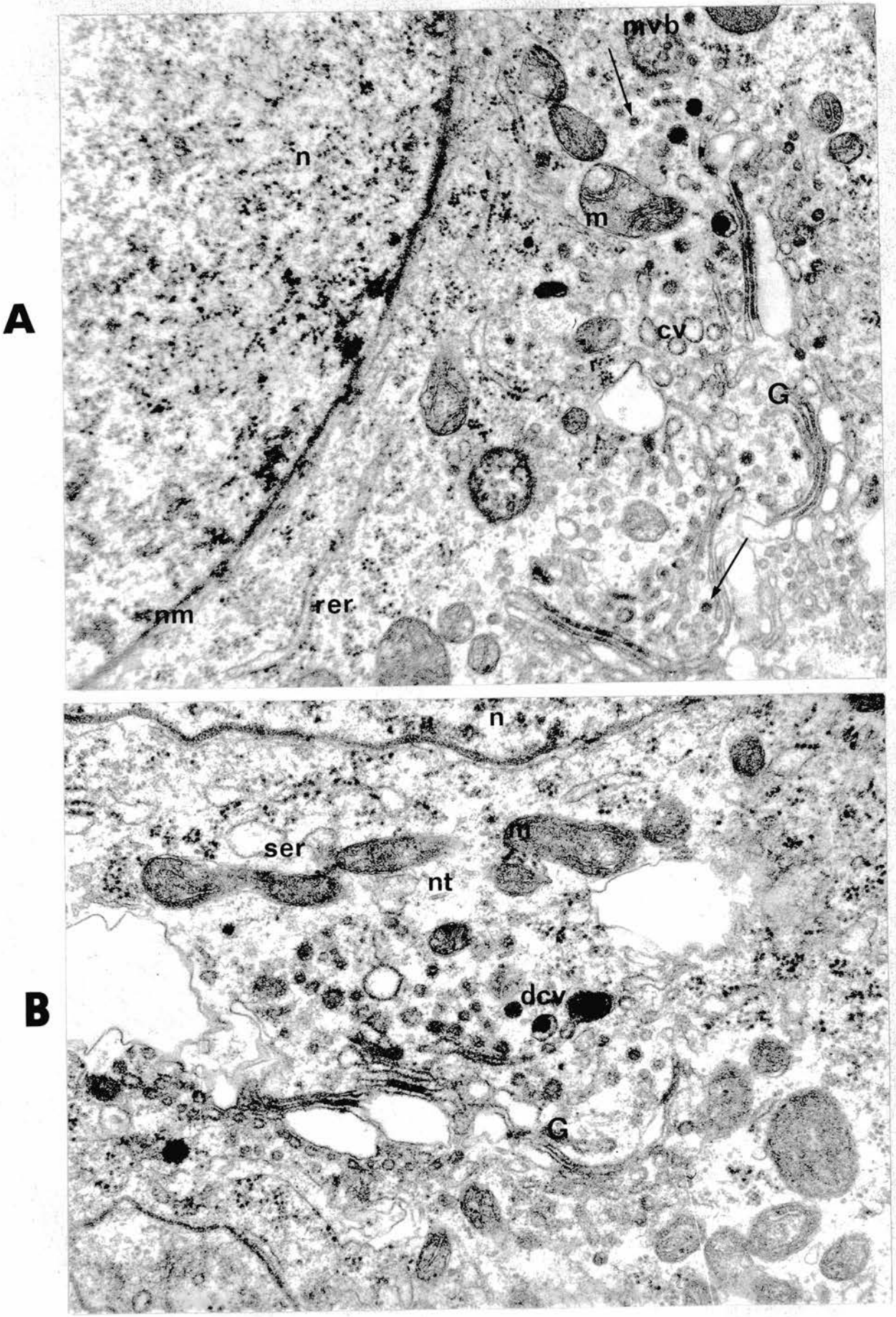
No count has been made of the ingluvial neurones though it is likely that they number hundreds rather than thousands, making the ganglion intermediate in size between the decapod crustacean stomatogastric ganglion and insect central ganglia.

In section the neurone somata are frequently pear- or spindle-shaped due to compression by neighbouring cells, with average dimensions of ca. 25 x 20 μm. Their nuclei generally are round in section, of 12 μm average diameter, and have prominent nucleoli (Figs. 7A, B). The nucleus is enclosed by a double membrane, whose inner layer is thicker and more densely stained than the outer layer (Figs. 8, 10B).

Mitochondria are abundant in the cytoplasm, frequently occurring in groups, especially near Golgi bodies (Figs. 10A, B). Numerous smooth-surfaced vesicles and tubules (Fig. 10B) of varying size probably represent elements of the agranular endoplasmic reticulum. Elements of the rough endoplasmic reticulum, with attached ribosomes are easily recognized (Figs. 9B, 10A, B). Free ribosomes also occur throughout the cytoplasm, generally in groups (Figs. 9B, 10A, B). Occasional neurotubules are seen (Figs. 9B, 10A, B).

Golgi bodies are conspicuous in the cytoplasm and several may generally be seen per cell in a section. They show the usual concentric arrays of paired membranes together with sacs and vesicles, and are frequently semi-circular in section (Fig. 9B). The vesicle population associated with the Golgi bodies includes unstructured clear vesicles of varying size, coated vesicles, multivesicular bodies (Fig. 10A) and dense-cored membrane-limited vesicles (dcv). The multivesicular bodies may be lipochondria

Figure 10. A and B. Electron micrographs of the ingluvial ganglion of Locusta migratoria showing parts of two neurone cell bodies. In each a Golgi body may be seen with densely-staining material within the channels and with several associated dense-cored vesicles (dcv). Groups of mitochondria occur near the Golgi bodies. In addition to dcv other vesicle types occurring at the Golgi apparatus are clear vesicles (cv), coated vesicles (arrows) and multivesicular bodies (mvb). In B a dense-cored vesicle is seen perhaps separating from one of the Golgi channels. Magnification A x 29,800, B x 36,800. G - Golgi body; m - mitochondrion; n - nucleus; nm - nuclear membrane; nt - neurotubule; rer - rough endoplasmic reticulum; ser - smooth endoplasmic reticulum.


A
mvb
n
m
cv
G
nm
rer
B
n
ser
nt
dcv
G

(see Lane 1968). The clear vesicles probably form part of the Golgi structure, and the coated vesicles may be involved in the interchange of materials between the Golgi apparatus and the rough endoplasmic reticulum (Odhiambo 1969).

No typical neurosecretory cells with cytoplasm filled with elementary neurosecretory granules were seen in sections of the ingluvial ganglion but since large numbers of neurones were not observed in section, it cannot be stated with certainty that they are absent. Other evidence suggests that the ganglion lacks cells containing typically-staining NSM (Delphin 1963; Strong 1966). However small numbers of dcv are observed near the Golgi bodies and occasionally in the cytoplasm (Figs. 10A, B). They are of approximately the same size range as dcv which occur in fibres of the neuropile. The dcv may occasionally be seen budding off from channels of the Golgi bodies and densely-stained material may sometimes be observed within the channels (Figs. 10A, B).

Small extensions of the neurone somata seen in section, are probably where axons arise. In such regions the volume of cytoplasmic contents decreases (Fig. 8). Nerve fibres cut in transverse section near neurone somata are probably axons directed towards the neuropile (Fig. 8).

Neuropile

In S. gregaria an extracellular layer between cortex and neuropile was noticeable in some regions of the ganglion (Fig. 8) and may correspond to the 'glial lacunar system' described by Wigglesworth (1960) in Periplaneta. This layer was not apparent in sections from L. m. migratorioides (see Fig. 9A). This may mean either that the layer is incomplete within the ganglion, that there is a species difference, that the extent of the system varies in different animals at different times, or that it reflects the different

fixation techniques used for the two species. Axon processes in this extracellular layer (Fig.8) are probably entering the neuropile.

The neuropile has a typically complex structure with fibres of wide-ranging diameter sectioned obliquely, transversely and longitudinally (Figs. 11, 12A,B). The largest fibres are probably axons or collaterals and the smallest probably represent terminal ramifications. The sheathed fibres seen at the left of Figure 12B, at the perimeter of the neuropile, may be entering the neuropile or may be part of a fibre tract leaving or passing through the ganglion. Within the neuropile some fibres are separated by thin layers of glial cytoplasm (e.g. fibres in centre of field, Fig.13A), but these are absent in many areas (left and right of field, Fig. 13A) and there is close apposition between adjacent fibres. While this provides innumerable sites for some kind of fibre interaction, morphological synapses characterized, amongst other features, by presynaptic clusters of small clear vesicles and thickened membranes 10 - 15 nm apart, were observed relatively infrequently. Probable synaptic regions are indicated by arrows in the low power micrographs of Figures 11 and 12.

As seen in Figures 11 - 13, the contents of fibres of the neuropile vary. Some of the smaller fibres in Figure 11 contain only mitochondria and neurotubules, while others presumably at or near their terminals, also contain vesicles of one or more types. Small clear synaptic-type vesicles within the sizerange 18 - 84 nm diameter in S. gregaria and 250 - 710 nm in L. m. migratorioides occur in large numbers. They may occur alone in fibres or mixed with dcv (see Figures 11 - 13) within the size range 40 - 250 nm diameter (with a very few up to 300 nm). The degree of intermingling when both types of vesicle are present varies and sometimes distinct segregation is apparent. Occasional fibres are seen where dcv

Figure 11. Electron micrograph of part of the neuropile of the ingluvial ganglion in Schistocerca gregaria, showing the complexity of structure and the range of fibre sizes and contents. Fibres are sectioned transversely, longitudinally and obliquely. Asterisks indicate where glial cytoplasm separates fibres - it is absent between many others. The arrow points to a probable synaptic region. Magnification x 17,000. F - fibre with dense-cored vesicles; lcv - large clear vesicles; m - mitochondrion; nts - neurotubules; scv - small clear synaptic-type vesicles.

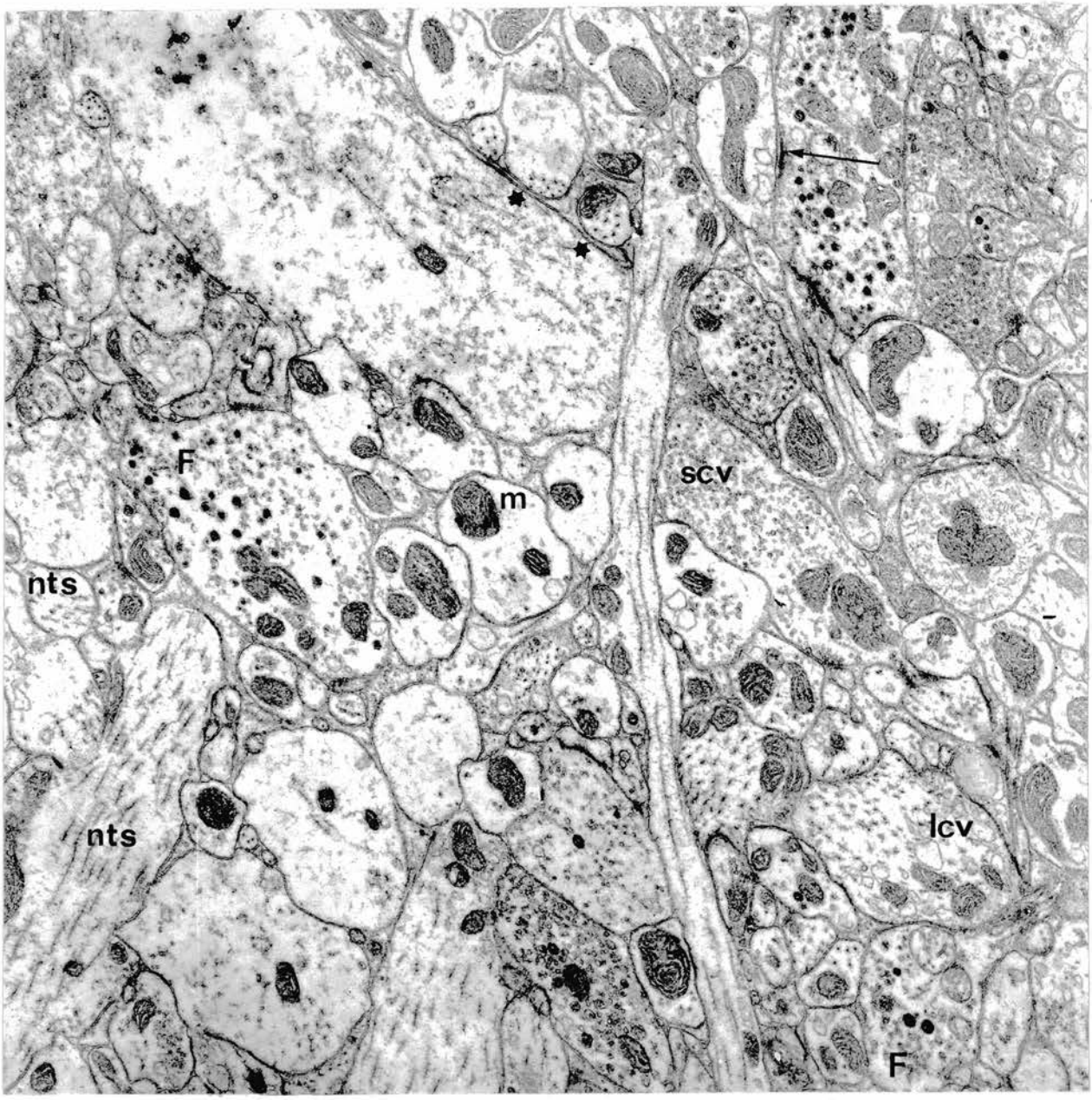
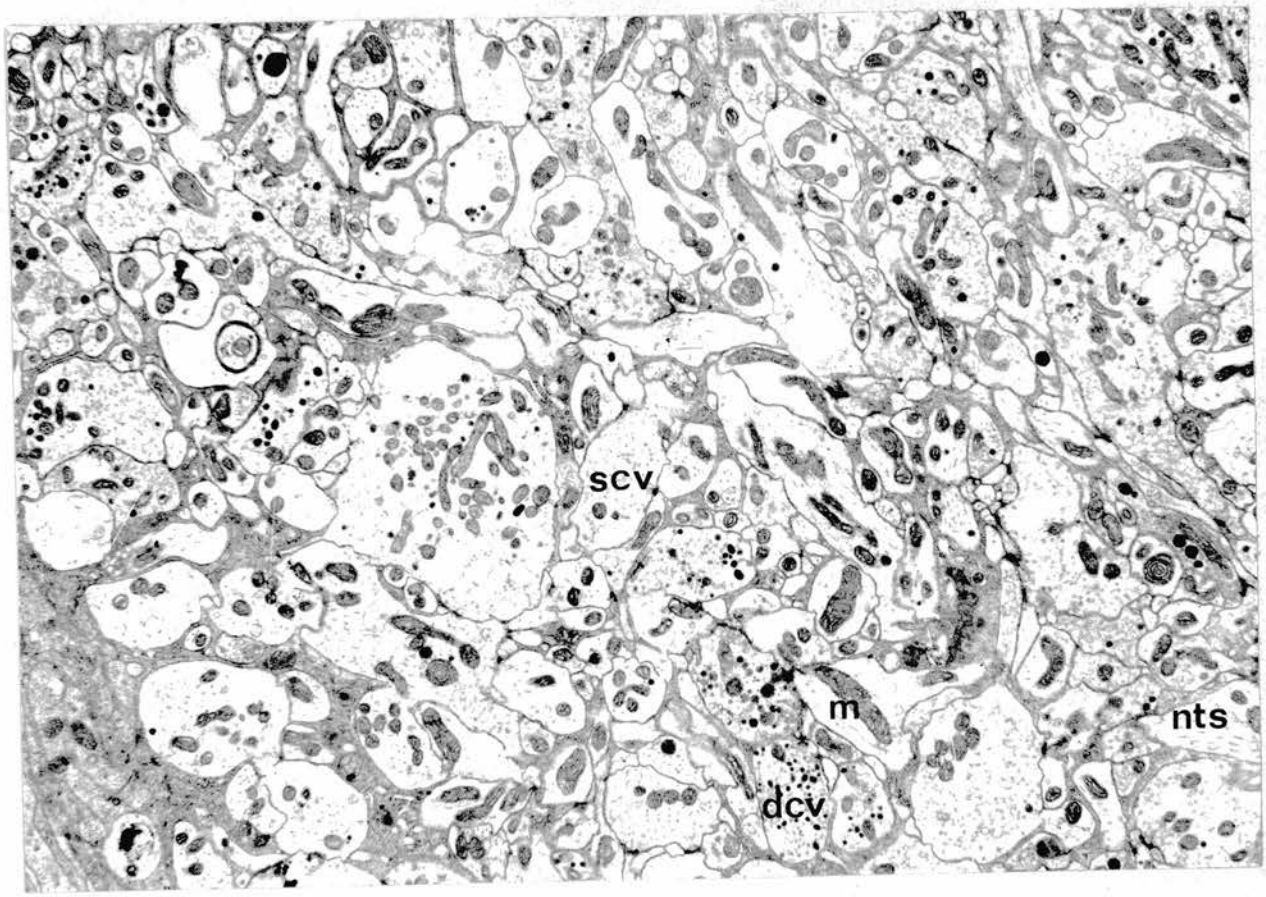

F
m
scv
nts
nts
lcv
F

Figure 12. A and B. Electron micrographs of the neuropile of the ingluvial ganglion in Locusta migratoria showing the range of fibre sizes and contents. The large sheathed fibres to the left of the field in B may be directed towards the neuropile or may be part of a fibre tract entering or leaving the ganglion. Also in B, there is considerable variability in different fibres of the size and staining properties of dcv. Arrows indicate probable synaptic regions. Magnification A and B x 7,500. ax - axon; dcv - dense-cored vesicles; gc - glial cytoplasm; lcv - large clear vesicles; m - mitochondrion; nts - neurotubules; scv - small clear synaptic-type vesicles.


A
scv
m
nts
dcv

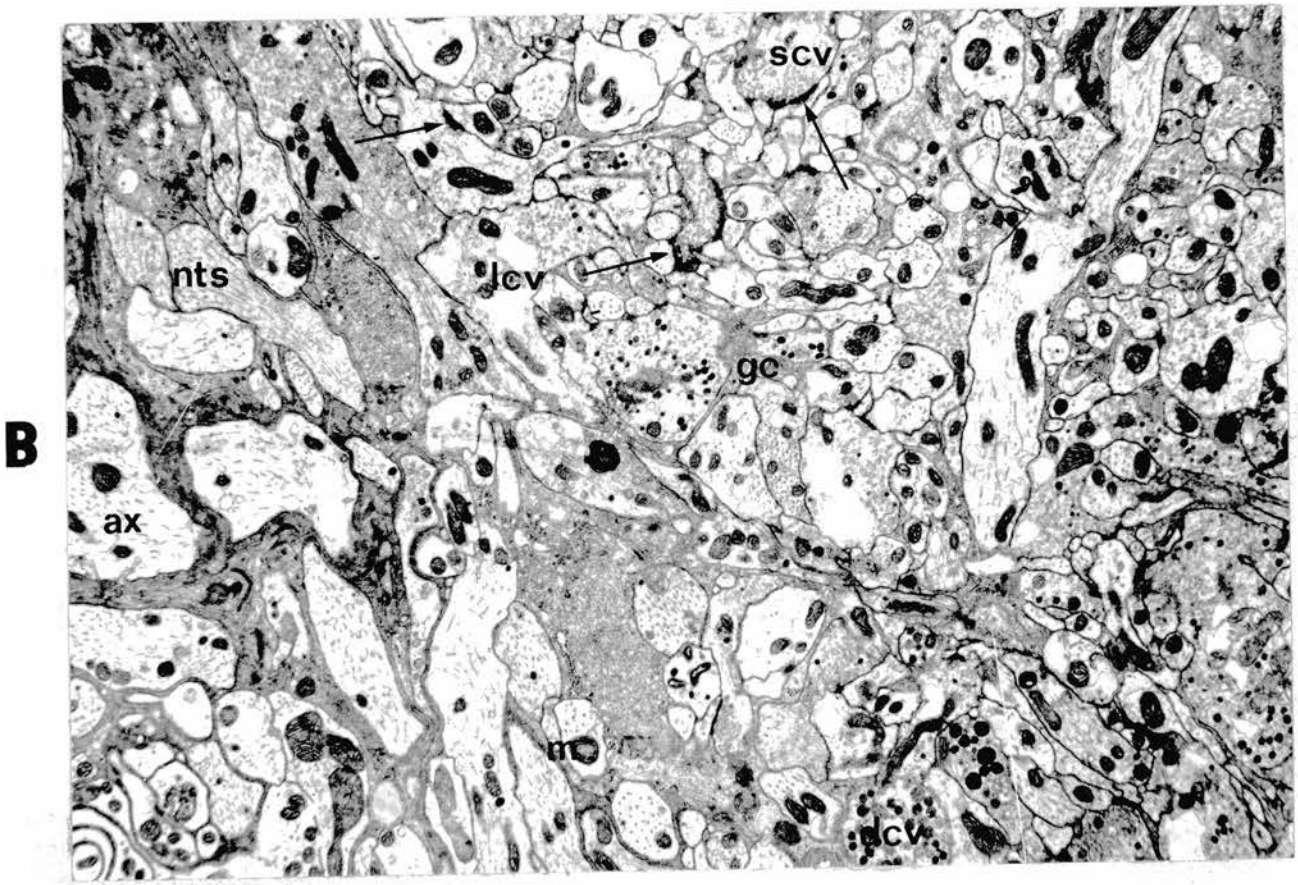
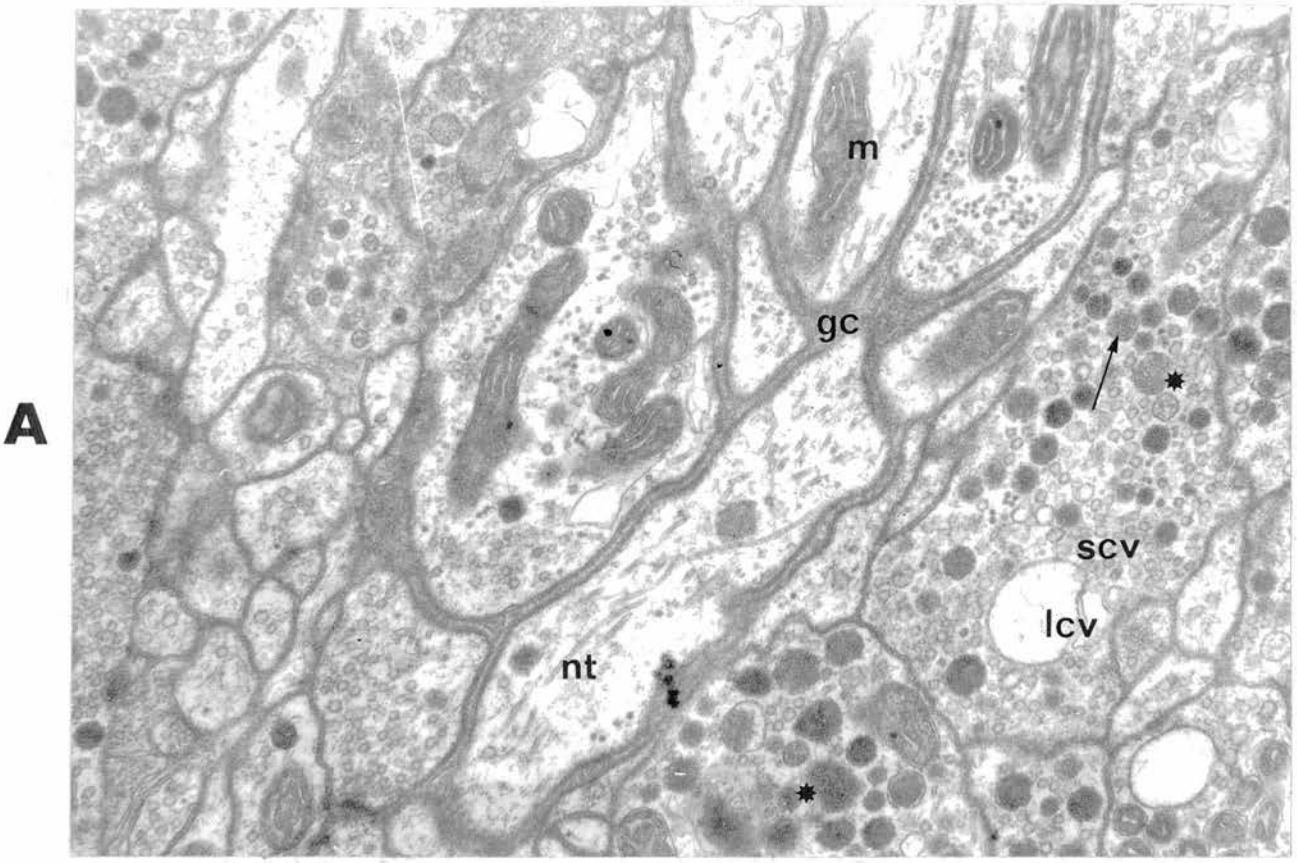
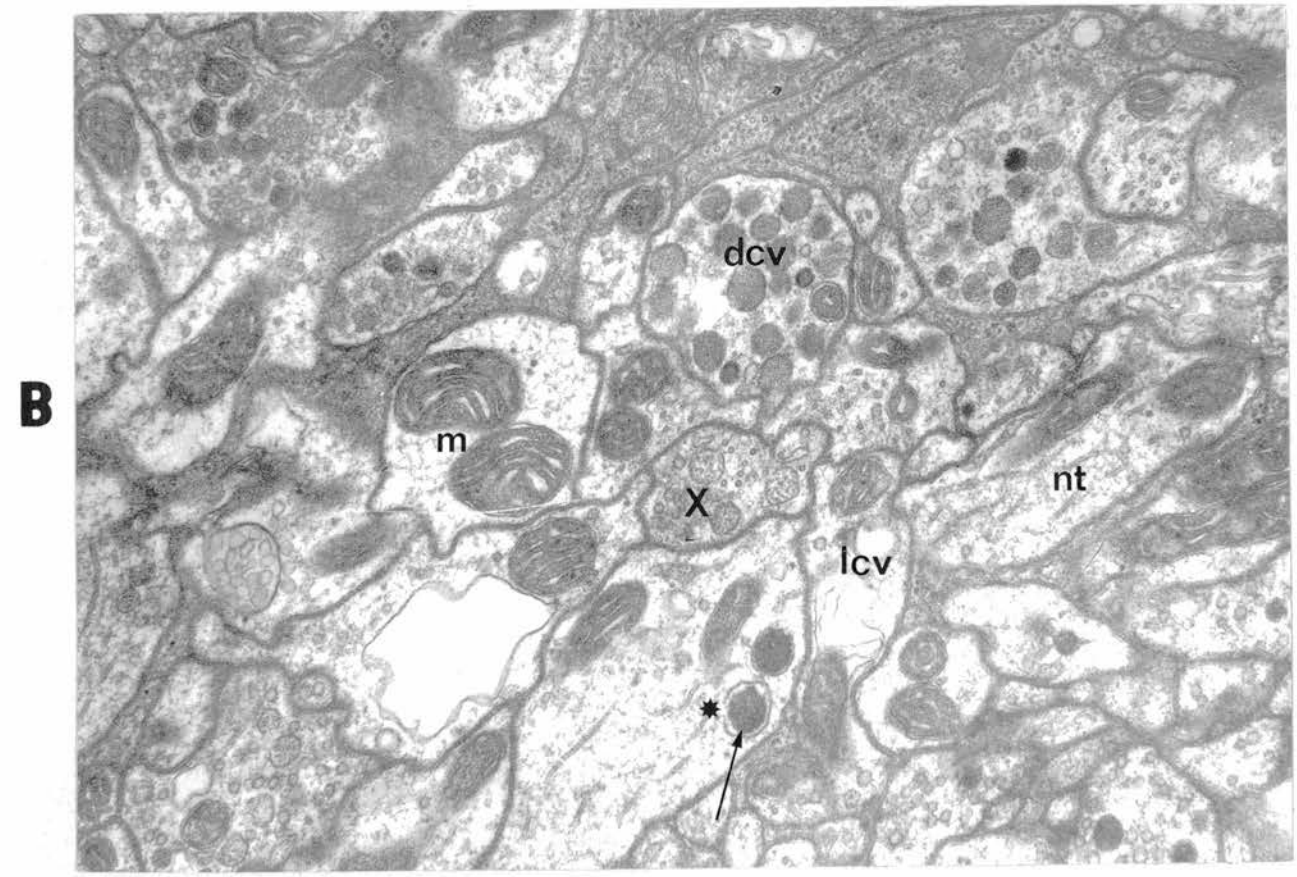

B
scv
nts
lcv
gc
ax
m
dcv

Figure 13. A and B. Electron micrographs of the ingluvial ganglion neuropile in Locusta migratoria. Many fibres contain small clear synaptic-type vesicles (scv), frequently together with variably-sized dense-cored vesicles (dcv). In some fibres the latter predominate. Some dcv appear to have finely granulated contents (arrows), others have fractured or indistinct membranes (asterisks) while the large vesicles in fibre X(B) may be dcv which have lost the bulk of their contents. Fibres in the centre of the field in A are separated by glial cytoplasm, those to the left and right are in close apposition. Magnification A and B x 28,200.. gc - glial cytoplasm; lcv - large clear vesicles; m - mitochondrion; nt - neurotubule.


A
m
gc
scv
lcv
nt


B
dcv
m
X
nt
lcv

predominate (Fig. 13A). The dcv were observed in material fixed in osmium tetroxide alone or in glutaraldehyde followed by osmium tetroxide, and in L. m. migratorioides the dense core was still present when double staining was omitted. In appearance, the dcv seen in electron micrographs resemble elementary neurosecretory granules described in the insect (Maddrell 1967) and many other nervous systems, and also those granules believed by many workers to contain or be capable of storing CA's and 5-HT (see for example Cottrell 1967; Tranzer and Thoenen 1967, 1968; Hökfelt 1969; Cottrell and Osborne 1970).

In many but not all instances the dcv may be seen to be membrane-limited (Fig. 13). The same figure also shows that occasionally the vesicle outline may be indistinct. All types of dcv (with or without visible membrane, or with indistinct outline) may occur in one fibre. The membranes of some dcv may be ruptured (Fig. 13B) but whether this is due to the fixation technique or some other cause is not known.

The intensity of staining of dcv cores varies within and between fibres (Figs. 12A, 13A, B) and the core sometimes appears to be finely granulated (Fig. 13B). Some almost clear granules with grainy contents may be dcv almost depleted of contents (Fig. 13B). Fibres in the neuropile of the ingluvial ganglion have not been classified into different types on the basis of dcv diameters, because of the wide size range that may occur within a single fibre.

Large unstructured, irregularly-shaped vesicles are frequently present in fibres (Figs. 11, 13). Their nature is unknown.

A short description of the fine structure of the outer oesophageal and ingluvial nerves of S. gregaria appears elsewhere (Dando 1968). Few fibres in these nerves were observed to contain dcv. Where they did

occur, they were present in small numbers (see Fig. 14 and Dando 1968).

Homarus - stomatogastric ganglion

This ganglion differs from the typical invertebrate ganglion in having most of its 30 or so neurones arranged in a dorsal rind over a ventral neuropile, rather than as a cortex round a central neuropile (see Fig. 15A). The neuropile is continuous anteriorly with nsgs and posteriorly with dvn. These nerves and the ganglion are enclosed in a connective tissue sheath which is thinner dorsally than ventrally and particularly over the neurone somata (Figs. 15A, B). The stomatogastric ganglion and normally also short adjacent lengths of nsgs and dvn, protrude through the ventral wall of the anterior aorta to lie within the lumen.

Glial cells

Layers of glial cytoplasm invest the neurone somata and may indent their cytoplasm (Fig. 16). Many of the fibres of the neuropile are also surrounded by glial cytoplasm (Figs. 20, 21). Such glial investment precludes the possibility of synapses in these regions. The nuclei of glial cells are small by comparison with neuronal nuclei, are irregularly-shaped and have densely-staining peripheral chromatin bodies.

Neurones

The somata are variably shaped in section (Fig. 15A) but under the dissecting microscope appear spherical to ovoid. The nuclei appear round to oval in section (Fig. 15A) and have prominent nucleoli. The nuclear/cytoplasmic ratio is apparently much smaller than in the locusts. As seen with the light microscope the neuronal cytoplasm has a fairly uniform appearance except for the presence of numerous crescentic bodies which probably represent the Golgi complexes (Fig. 15A).

Figures 16 and 17 show the neuronal cytoplasm at greater resolution.

Figure 14. Electron micrograph of a transverse section through the outer oesophageal nerve of Schistocerca gregaria. Note the range in fibre size. A few fibres (F) contain small numbers of dense-cored vesicles. Magnification x 19,000. em - extracellular material; gc - glial cytoplasm; m - mitochondrion; nts - neurotubules.

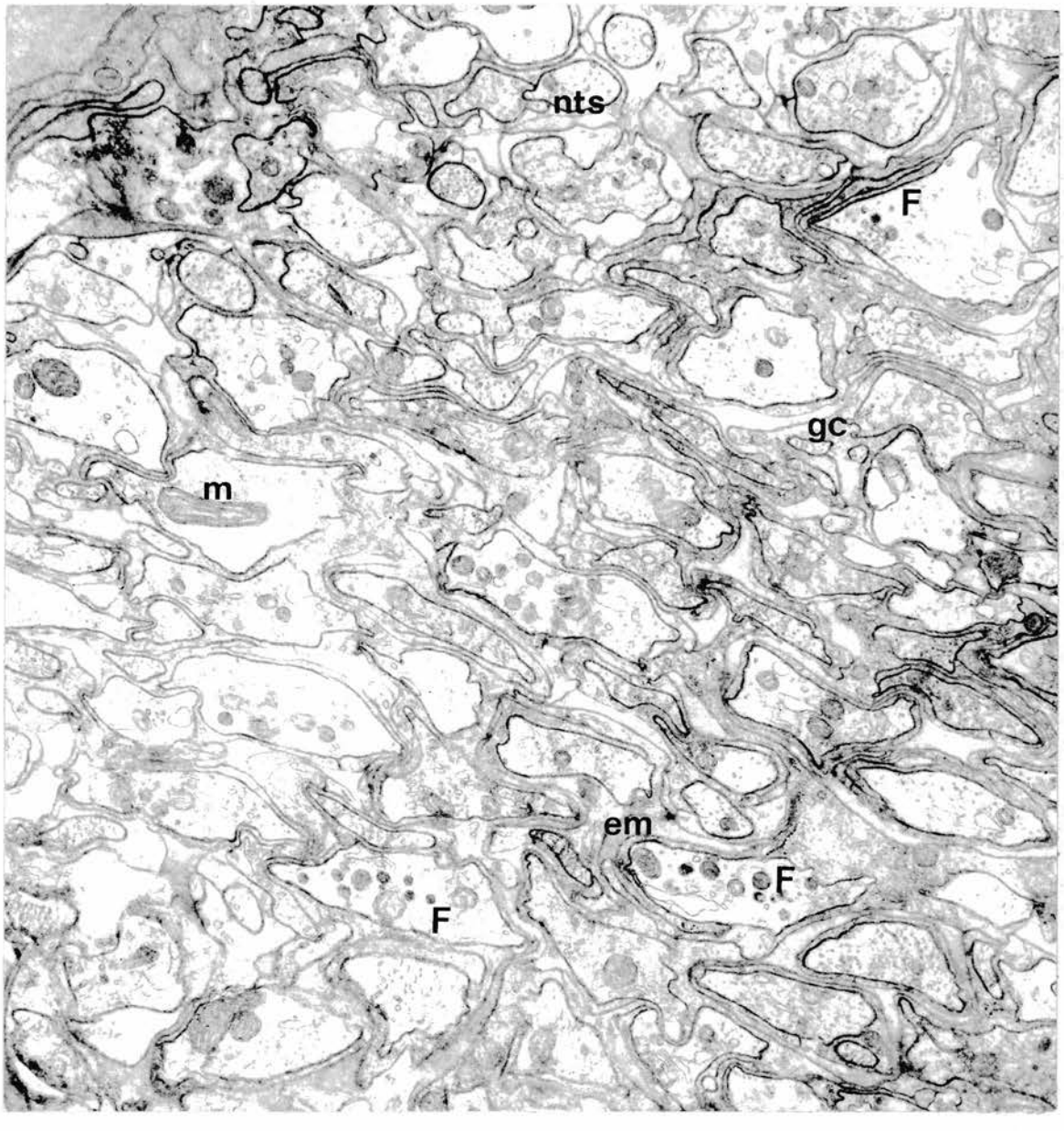
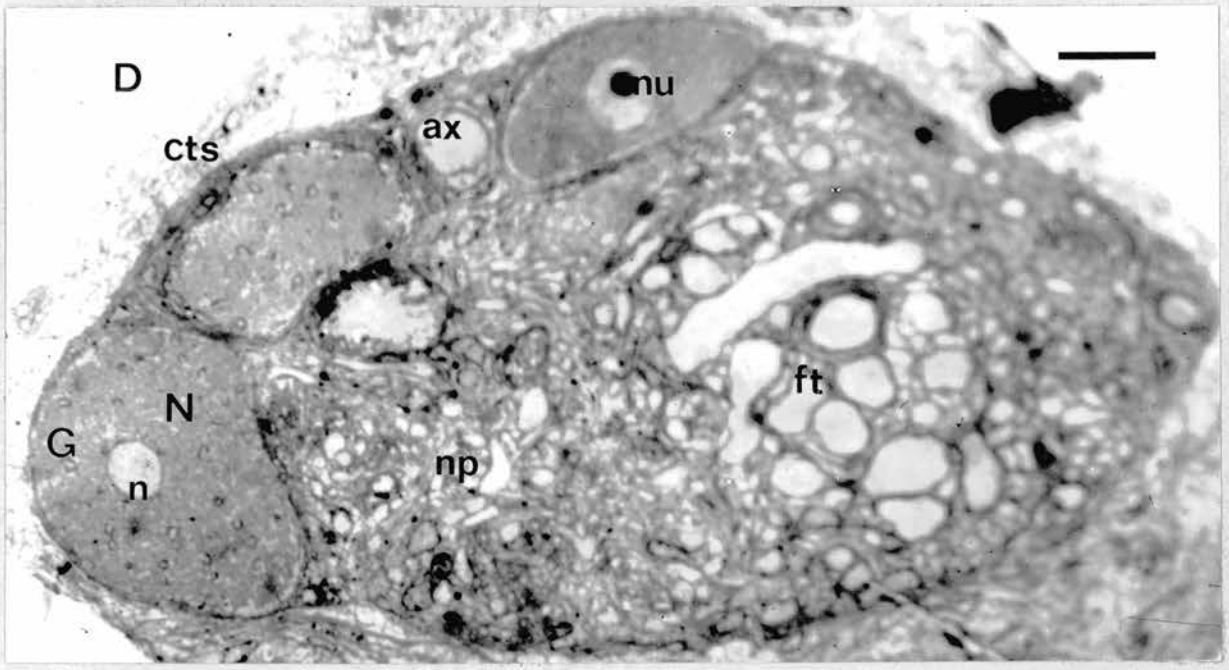
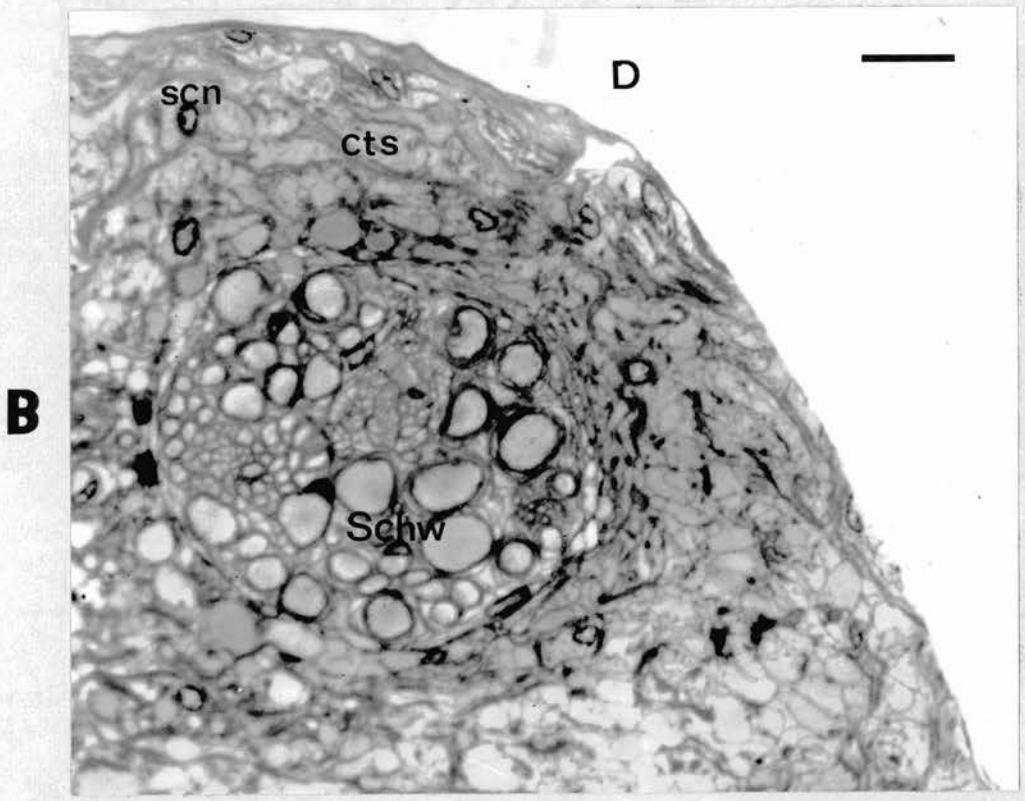

nts
F
gc
m
em
F
F

Figure 15. A. Photomicrograph of a transverse section through the anterior end of Homarus stomatogastric ganglion (stained with toluidine blue). The ganglion is surrounded by a connective tissue sheath (cts) which is thinner dorsally over the neurone somata. The region of the ganglion below the somata has areas of true neuropile and others which are probably fibre tracts of the stomatogastric nerve. Scale mark 20 µm. B. Photomicrograph of a transverse section through the stomatogastric nerve of Homarus gammarus. The thin part of the connective tissue sheath (cts) is dorsal. In the nerve groups of fibres of similar size may be seen which appear to be ensheathed. Scale mark 20 µm.
ax - axon; D - dorsal; ft - fibre tract; G - Golgi body; N - neurone soma; n - nucleus; nu - nucleolus; np - neuropile; scn - sheath cell nucleus; Schw - Schwann cell nucleus.


A
D
cts
ax
nu
ft
N
G
n
np


B
scn
D
cts
Schw

Definition is not as good as in the locust material and this may be due partly to a difference in fixation technique (osmium tetroxide alone being used). However the range of cytoplasmic contents is the same and includes mitochondria, rough and smooth endoplasmic reticulum, ribosomes, neurotubules, Golgi complexes and large densely-stained bodies associated with them. The Golgi bodies are composed of concentric paired membranes and vesicles of different types and sizes, and mitochondria are closely associated with them. In Figure 17 one or two dcv are located near the Golgi body and there may be electron-dense material in one of its channels.

Neuropile

Figure 15A is a micrograph of a section cut from the anterior end of the stomatogastric ganglion. The ventral region of the ganglion has areas which are true neuropile and others which are probably fibre tracts from nsgs. High power micrographs of the neuropile region show a complex array of fibre types and sizes (Fig. 20) and it is not always easy to distinguish fibres from glial cytoplasm. Many of the fibres in Figure 20 contain only mitochondria and neurotubules but others, which are often grouped (see Figs. 20, 21), contain numerous membrane-limited dcv generally together with small clear synaptic-type vesicles. In such fibres mitochondria are few and neurotubules are not readily apparent.

The small clear vesicles range from 23 - 56 nm in diameter. The dcv range from 33 - 140 nm in diameter. The latter show considerable variability in size, intensity of staining of the core, and membrane/core separation even within one fibre (see Fig. 21). The core of some of the dcv appears to be structured (arrows, Fig. 21). A fibre may contain small clear vesicles, occasionally dcv only, but most often a mixed population of the two types. In the mixed populations one type may predominate, and though

Figure 16. Electron micrograph of part of a neurone from the stomatogastric ganglion of Homarus gammarus, probably in the region of the axon hillock where the volume of cytoplasmic organelles decreases. Layers of glial cytoplasm surround the neurone and glial processes indent its cytoplasm (arrow). The nature of large densely-staining bodies near the Golgi body (asterisks) is not known. Magnification x 18,750.
G - Golgi body; gc - glial cytoplasm; m - mitochondrion; nt - neurotubule; r - ribosomes; rer - rough endoplasmic reticulum; ser - smooth endoplasmic reticulum.

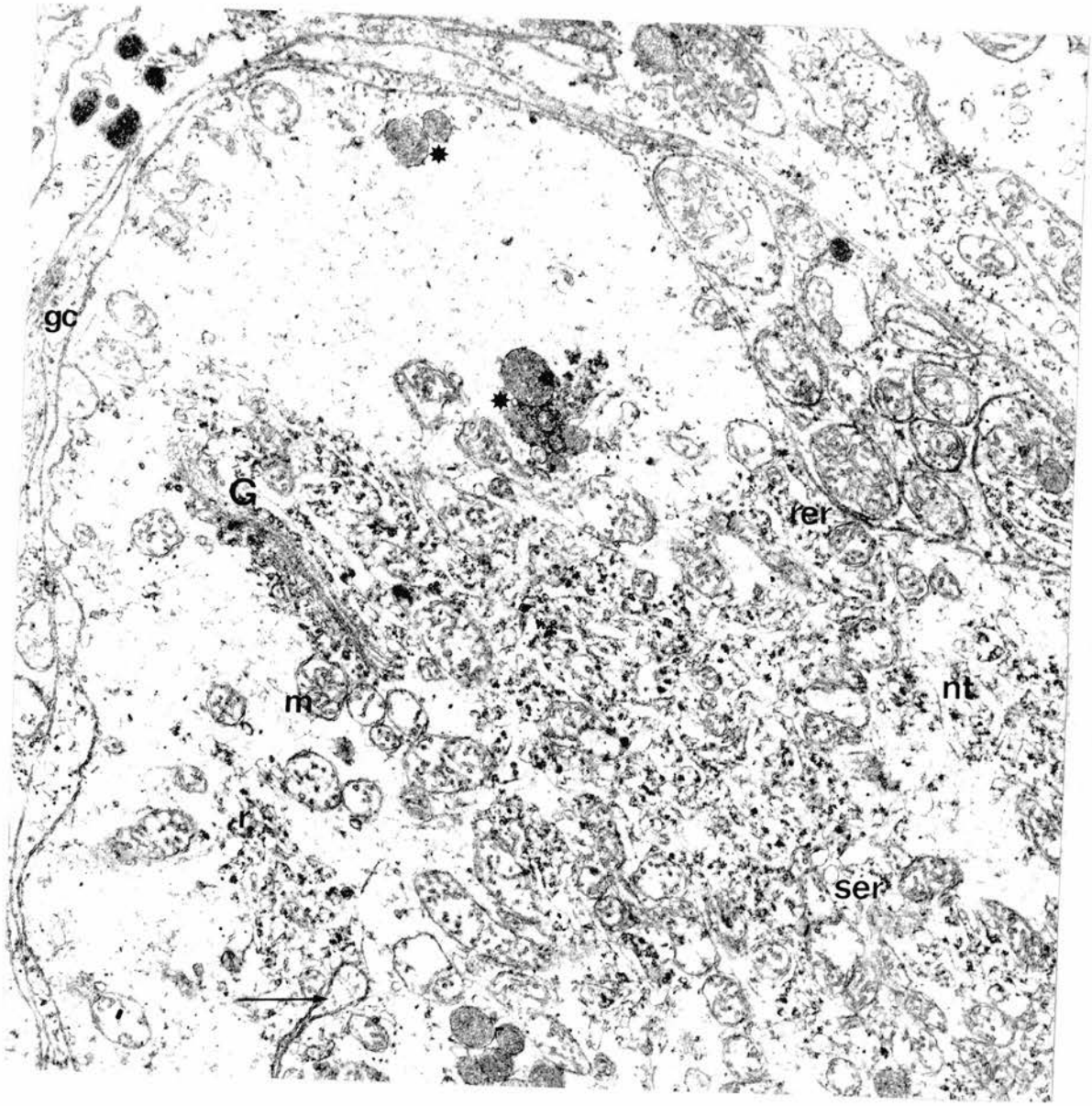

gc
G
rer
nt
m
r
ser

Figure 17. Electron micrograph of part of a neurone from the stomatogastric ganglion of H. gammarus, showing a Golgi body with paired membranes and many vesicles. Arrows indicate one or two dense-cored vesicles. Magnification x 42,000. G - Golgi body; m - mitochondrion; nt - neurotubule; r - ribosomes; rer - rough endoplasmic reticulum; ser - smooth endoplasmic reticulum.

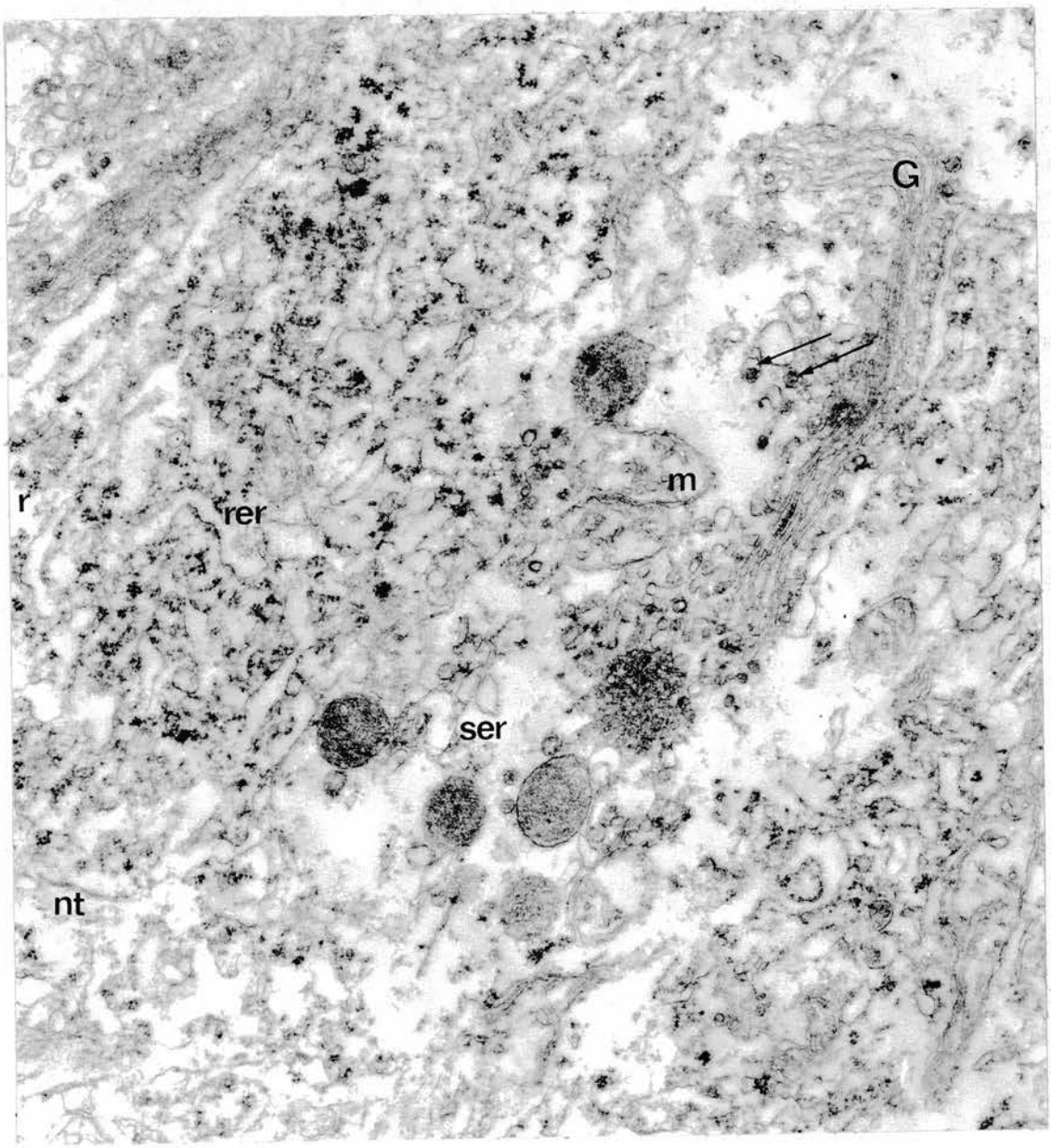

G
m
r
rer
ser
nt

Figure 18. Electron micrograph of a transverse section through the stomatogastric nerve of H. gammarus, showing some fibres of a small fibre group seen at lower power in Figure 15B. The small grouped fibres are encapsulated in glial cytoplasm (gc) and then extracellular material (em). Magnification x 24,000. cv - clear vesicles; m - mitochondrion; nts - neurotubules.

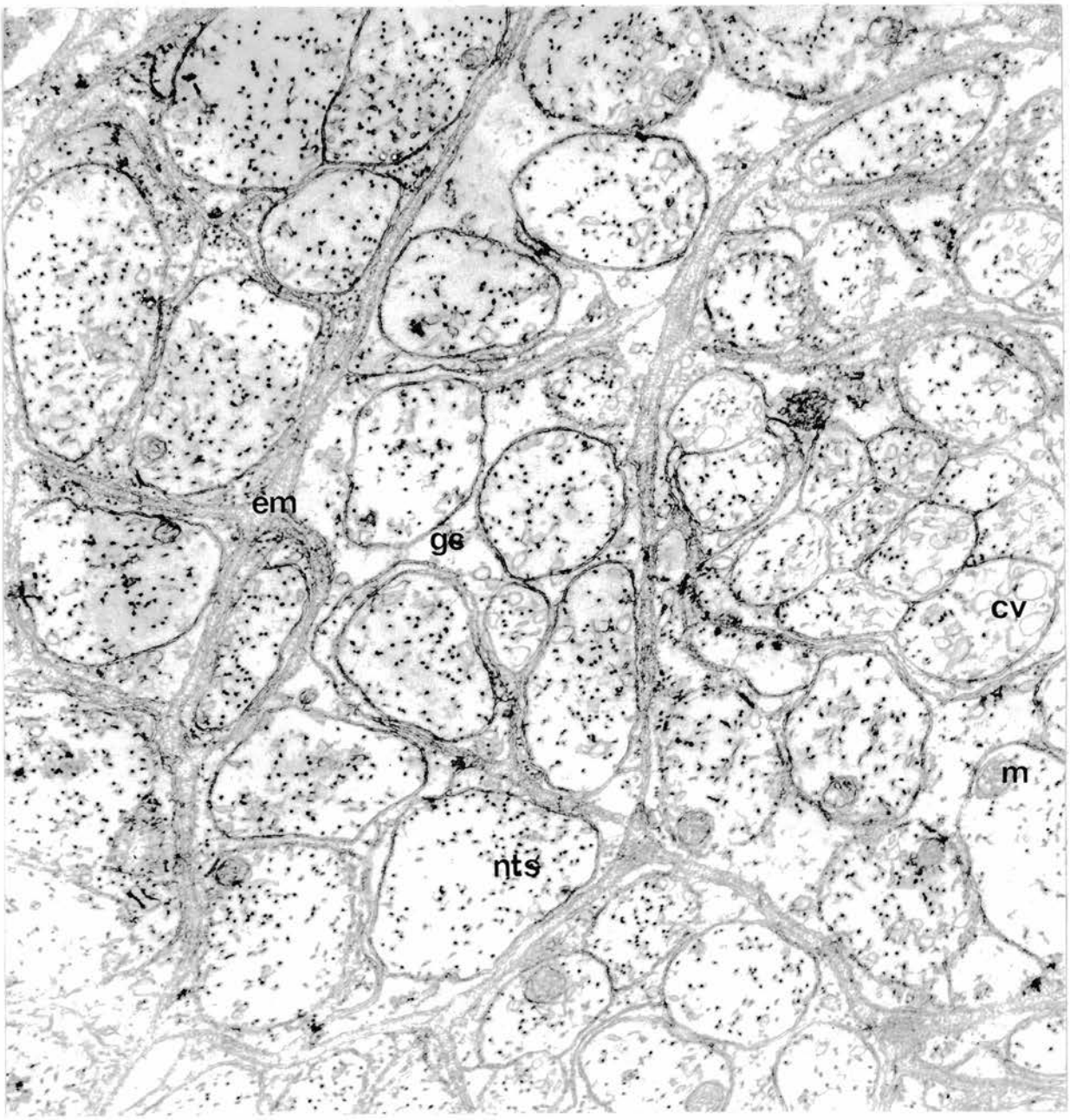

em
ge
cv
m
nts

Figure 19. Electron micrograph of a transverse section through the stomatogastric nerve of H. gammarus showing several fibres, some of which contain dense-cored vesicles (arrows). Magnification x 22,000. gc - glial cytoplasm; lcv - large clear vesicles; m - mitochondrion; nts - neurotubules.

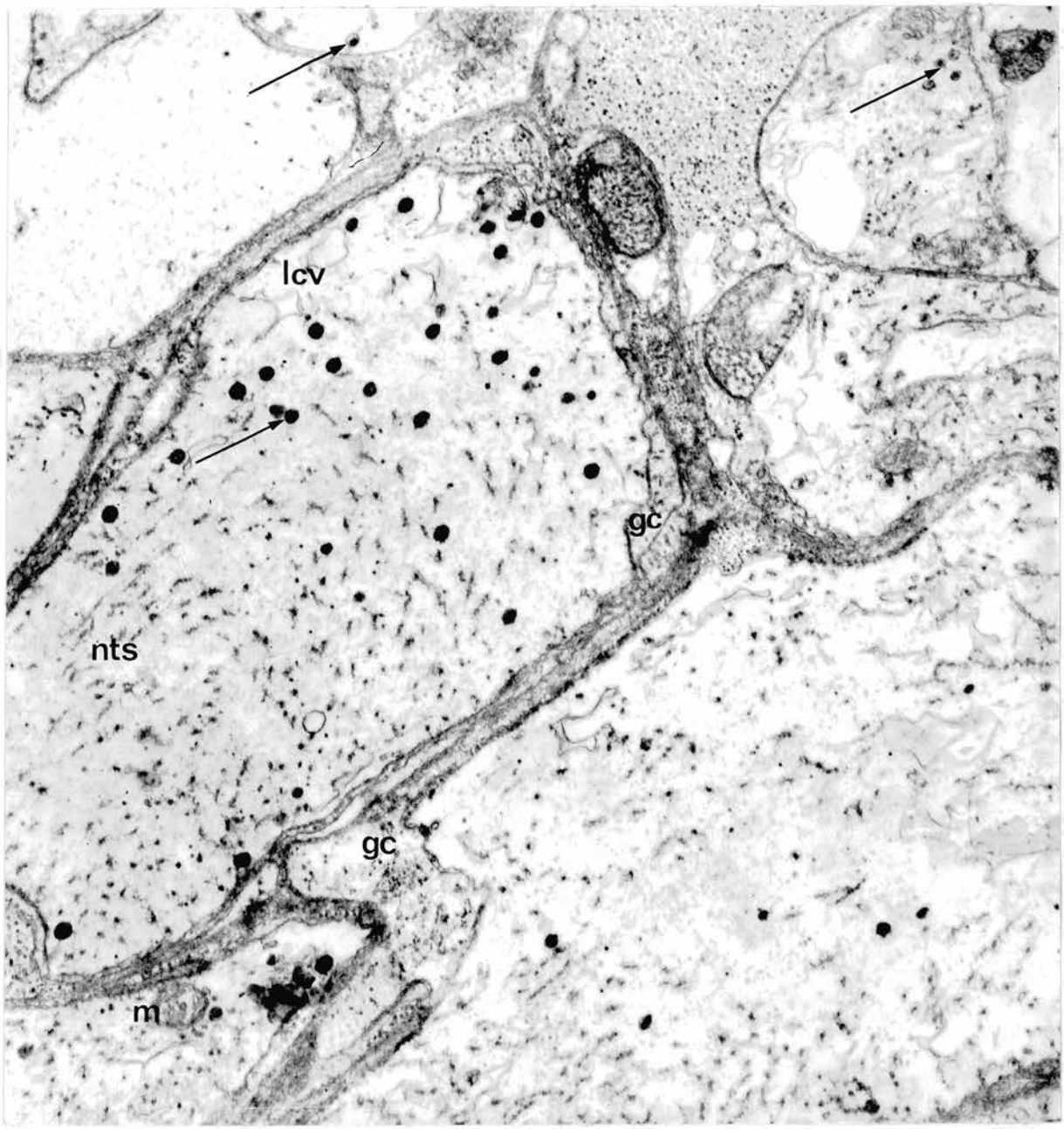

lcv
gc
nts
gc
m

Figure 20. Electron micrograph of a transverse section through the neuropile of the stomatogastric ganglion of H. gammarus. There is a group of small fibres at lower left of the field. Several larger fibres contain many dense-cored vesicles (dcv) as well as small clear synaptic-type vesicles (scv). The dense core of the dcv varies in size and there is considerable variability in membrane/core separation in different vesicles. Glial cytoplasm separates some fibres but is absent between others (arrows). Magnification x 18,750. gc - glial cytoplasm; lcv - large clear vesicles.

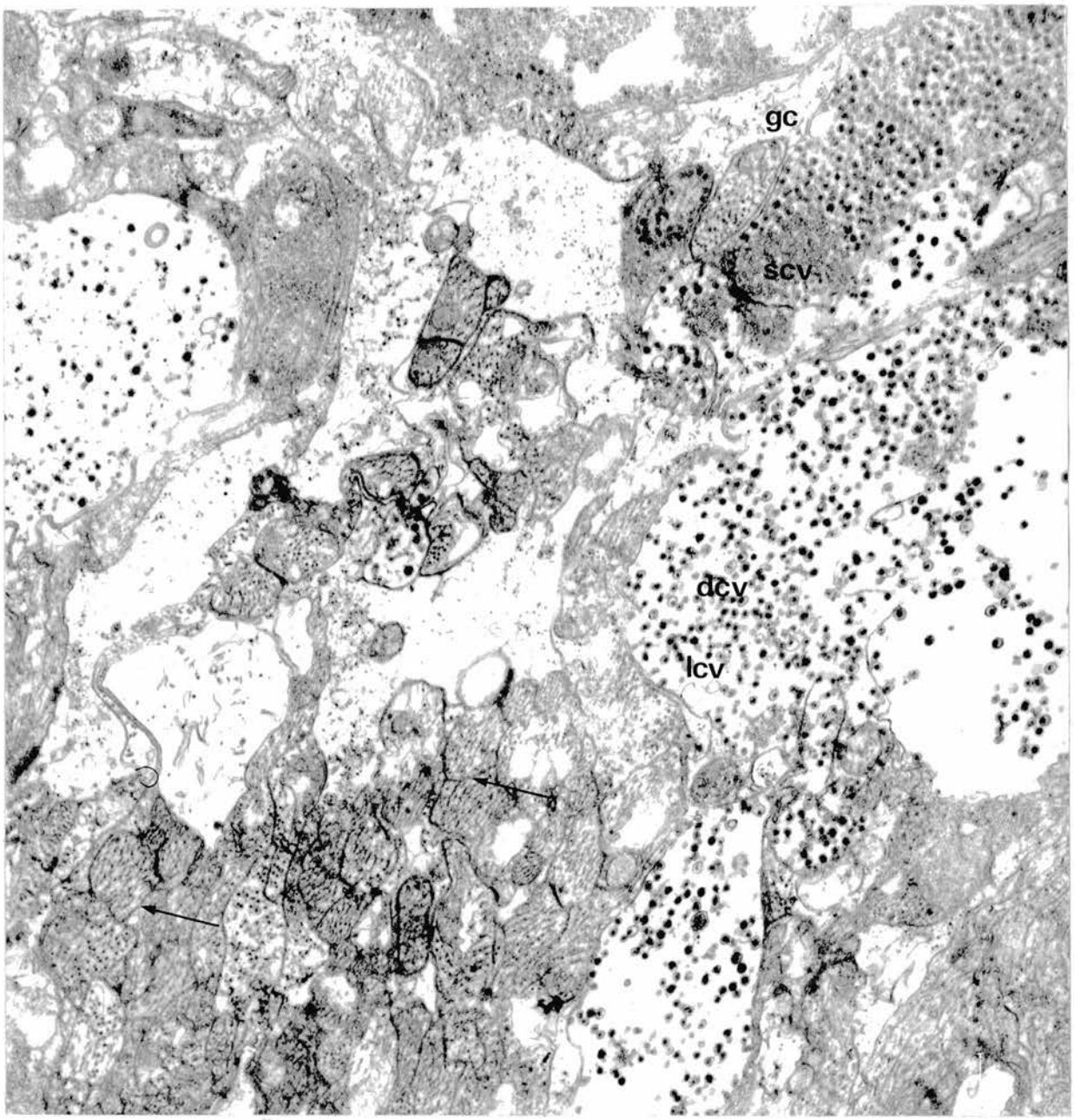
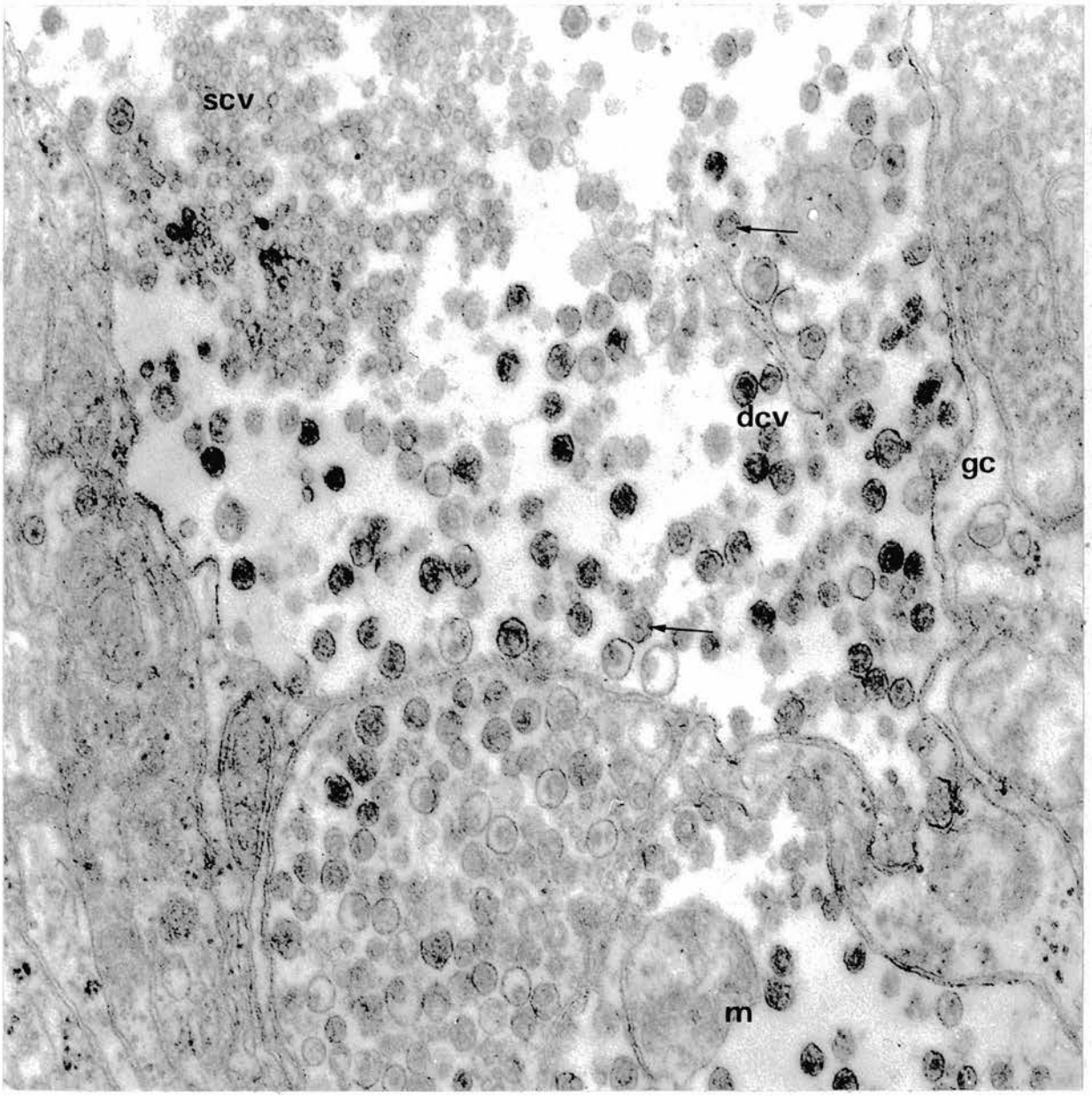

gc
scv
dcv
lcv

Figure 21. Electron micrograph of a transverse section through the neuropile of the stomatogastric ganglion of H. gammarus, showing fibres containing dense-cored vesicles (dcv) and small clear synaptic-type vesicles (scv). In these fibres mitochondria and neurotubules are few or not readily seen. Note the variability in size, density of staining and membrane/core separation of the dense-cored vesicles. Some may be internally structured (arrows). Magnification x 53,300. gc - glial cytoplasm; m - mitochondrion.


scv
dcv
gc
m

intermingling occurs, segregation according to type is often seen (Figs. 20, 21).

Morphological synapses were not observed in the neuropile though they must be present. They have been described by Maynard (1971a) in Homarus americanus. Tight junctions have been observed in the neuropile of H. americanus by Moulins (1971 personal communication).

Nerves

Most observations were made on nsgs and only one dvn was sectioned. Nsgs contains many fibres (approximately 240 in H. americanus, Maynard 1971a) of widely ranging diameter from about 10 µm down to 0.2 µm and less. The section shown in Figure 15B contains 15 fibres over 8 µm in their greatest diameter. As this figure shows, fibres of a similar size tend to be grouped together. The bundles of small fibres appear at low magnification to be enclosed in a sheath. At higher magnification Figure 18 shows that in the small fibre bundles the fibres, whether closely apposed to each other or surrounded by cytoplasm from a common glial cell, are further enclosed by a layer of fibrous extracellular material. This layer probably corresponds to the sheath apparent at low magnification.

The small fibres of nsgs contain occasional mitochondria, neurotubules, and clear often irregularly-shaped vesicles (Fig. 18). The small clear vesicles around the edge of these fibres are probably fixation artifacts caused by the membrane rupture which is apparent in parts of this section. This is unlikely to apply to all such vesicles however for they are also present in fibres in Figures 19 and 20 where the fixation procedure was different and the membranes are better preserved.

The large fibres of nsgs are individually ensheathed in one or more layers of glial or Schwann cell cytoplasm and the crescentic nucleus

of the Schwann cell is frequently visible in sections (see Fig. 15B). The mitochondria generally have a peripheral distribution in the fibres and neurotubules are prominent.

In a few fibres of nsgs (not the large fibres) small numbers of dcv are seen, with or without a visible limiting membrane (arrows, Fig. 19). These vesicles are seen both in material fixed in osmium tetroxide alone and in glutaraldehyde plus osmium tetroxide, but they are better preserved by double fixation. Their size range is 44 - 135 nm, thus corresponding approximately to the range for dcv in the ganglion neuropile.

3. Falck and Hillarp Technique - localization of biogenic amines.

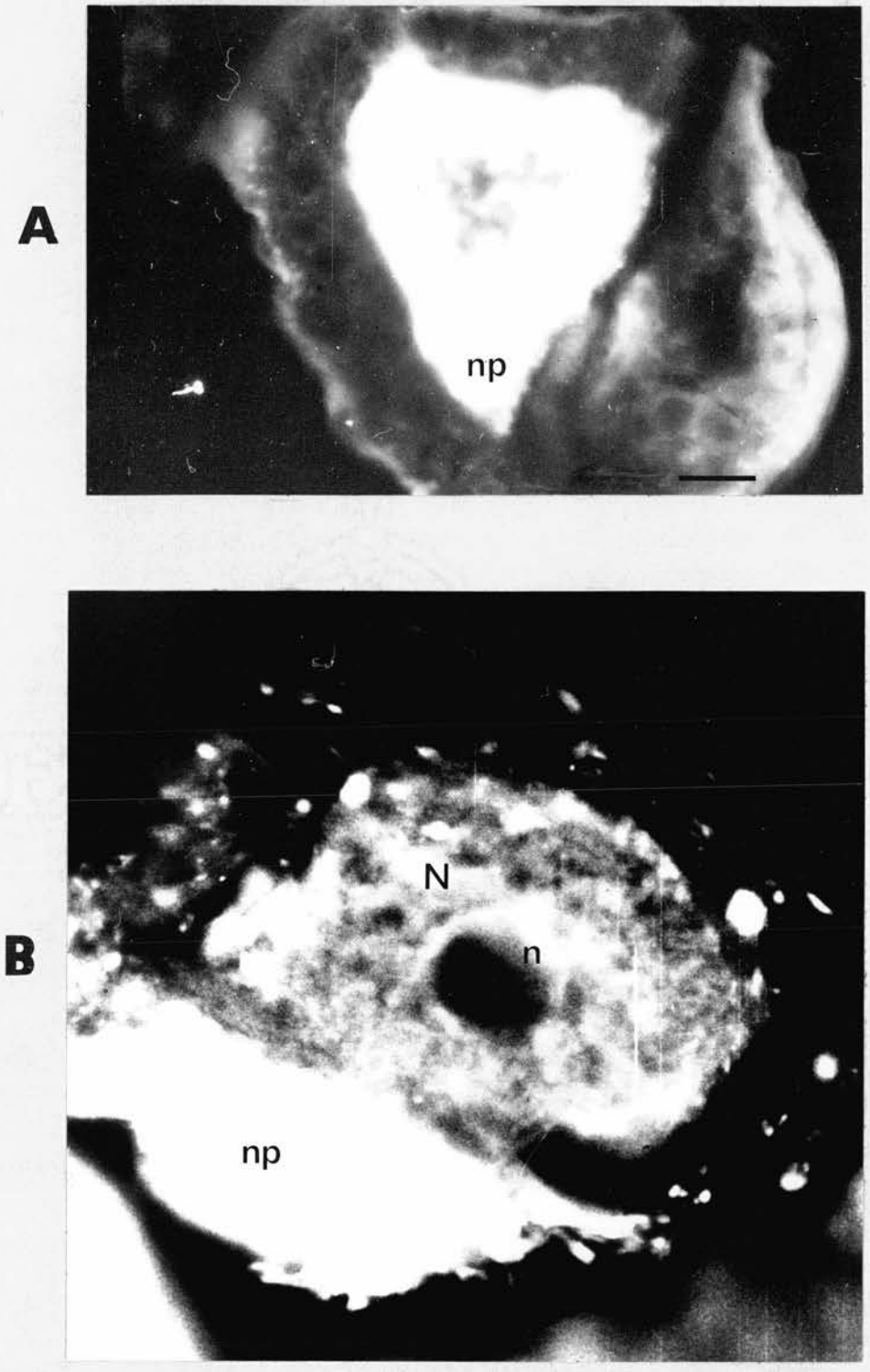
Ingluvial ganglion of S. gregaria

In sections of the ganglion treated with formaldehyde vapour a strong greenish-yellow fluorescence was seen in the region of the neuropile. The fluorescence sometimes had a granular appearance, at other times it appeared more diffuse (as in Fig. 22A). The strong fluorescence was absent from the rest of the ganglion though some yellow fluorescence was apparent in the sheath and as a few bright spots around the neurones. The yellow fluorescence was also present in control ganglia and therefore can be assumed to have been autofluorescence. In Figure 22A the neuronal nuclei appear somewhat darker than the cytoplasm, but no intense specific fluorescence was seen in the neurones.

In sections from control ganglia, not exposed to formaldehyde vapour, the greenish-yellow fluorescence was absent from the neuropile though a low level of background fluorescence was observed (autofluorescence).

During prolonged exposure to ultra-violet irradiation formaldehyde-treated sections of the ganglion showed a noticeable reduction in intensity of the green-yellow fluorescence. Formaldehyde-treated sections which were

Figure 22. A. Photomicrograph of a longitudinal section through the ingluvial ganglion of Schistocerca gregaria, after treatment with formaldehyde vapour. The neuropile (np) appears bright due to the presence of a green-yellow specific fluorescence indicative of biogenic amines. The neuronal somata, though they can just be distinguished in this micrograph, show no such bright fluorescence. Scale mark 50 µm. B. A section through the stomatogastric ganglion of Homarus gammarus after treatment with formaldehyde vapour (Osborne and Dando 1970). The section includes a neurone cell body whose cytoplasm showed green-yellow fluorescence indicative of the presence of a biogenic amine. The nucleus appears dark. Bright specific fluorescence was also present in the neuropile. Scale mark 20 µm. n - nucleus; N - neurone soma; np - neuropile.


A
np
B
N
n
np

immersed in alcoholic sodium borohydride solution also showed reduction of the fluorescence.

The specificity of the technique, the emission wavelength (i.e. colour) of the fluorescence, the reduction of the fluorescence in sodium borohydride solution and under prolonged U.V. irradiation, and the absence of strong fluorescence in control ganglia, lead to the conclusion that the green-yellow fluorescence seen in the neuropile of formaldehyde-treated ingluvial ganglia was specific fluorescence attributable to the presence there of a biogenic amine (s) (see Falck 1962; Corrodi and Jonsson 1967).

As to the identity of such an amine, a catecholamine is indicated rather than 5-HT since the colour of the fluorescence was green-yellow and not yellow, and the fluorescence faded relatively slowly under U.V. irradiation. Such fading is rapid when 5-HT is present. Because the fluorescence developed under relatively mild reaction conditions (one hour exposure to formaldehyde vapour at 80°c) it was most likely due to a primary, not a secondary CA (i.e. DA or NA or a mixture rather than A). DA and NA cannot be distinguished by the colour of their specific fluorescence alone but can only be differentiated by measuring the emission wavelengths of their fluorescence or by using biochemical and pharmacological tests (Corrodi and Jonsson 1967). To obtain a positive identification these procedures should be carried out. The method of Wood (1966) for the electron microscopic localization of amines in central nervous tissue and the argentaffin reaction (see later) used in conjunction with electron microscopy, might be appropriately used for the subcellular localization of biogenic amines in the ingluvial ganglion.

The occurrence of 5-HT in the neuropile of the ingluvial ganglion is not ruled out. If small amounts only were present and it had a similar distribution to DA and/or NA its fluorescence after formaldehyde treatment

could be masked by that of primary CA's.

IV. DISCUSSION

1. Nervous transmitters in arthropods

Several biologically active substances (see introduction) have been extracted from arthropod tissues. Their occurrence in whole body extracts is not necessarily evidence of a neurotransmitter status since they may have other physiological roles. DA, for example, is involved in the process of tanning of the insect cuticle (see Wigglesworth 1970). Even when biologically active substances are extracted from specific organs such as the brain, nerve cord, corpora cardiaca or pericardial organs there is often little indication of their role in the whole animal. However a considerable amount of evidence now accumulating, strongly implicates at least three of these substances, Ach, GABA and L-glutamic acid, in arthropod nervous transmission.

Ach

Central nervous tissues of the arthropod species investigated have shown high concentrations of Ach while the peripheral nerves and connectives have shown low concentrations (see Treherne 1966). In Cancer magister the total Ach content of cheliped nerves was accounted for by sensory fibres alone and Florey (1967) has suggested that all crustacean sensory neurones may be cholinergic. Maynard (1971b) found the principal cholinesterase reaction of the sensory fibres she examined in Homarus americanus to be in the sheath elements of the fibres, the fibres themselves showing no axoplasmic reaction. The cell bodies of these fibres were not analysed and their reaction could not be predicted on the basis of that in the fibre. Moreover since the presence of cholinesterase in neurones is not reliable for predicting the nature of their transmitter Maynard's evidence neither confirmed nor denied Florey's suggestion (Maynard 1971b). Crustacean

peripheral excitatory motor axons and the inhibitory fibres which have been investigated are non-cholinergic (see Maynard 1971b for references). In insects also the conventional cholinergic system is absent from skeletal muscle (see Treherne 1966).

Synthesis of Ach has been demonstrated in arthropod nervous tissue and cholinesterase activity has been found to be widely distributed, being particularly intense in neuropile regions (see Treherne 1966). Using the electron microscope Smith and Treherne (1965) localized acetylcholinesterase activity in insect neuropile to regions which included areas along axon branch membranes frequently in association with clusters of synaptic vesicles. Maynard (1971b) demonstrated moderate to strong cholinesterase activity in all synaptic regions of central ganglia and the stomatogastric ganglion of lobsters. It occurred in perineuronal glial sheaths, around peripheral axon sheaths and in the cytoplasm of a few neurone somata in central ganglia.

In the cockroach CNS, the only place in arthropods where the existence of a cholinergic system could be postulated with any certainty (Treherne 1966), a major difficulty to the acceptance of Ach as a transmitter was the high concentration needed to cause excitation when it was applied to ganglia and nerves. The reason suggested for this was the high concentration of cholinesterase in the insect nerve cord which reduced the effective concentration of Ach at the nerve membrane. Recently Kerkut et al. (1969) using intracellular recording techniques and direct iontophoretic application of Ach to nerve cells, have shown that cockroach central neurones are as sensitive to applied Ach as other neurones (e.g. in molluscs or vertebrates). Steiner and Pieri (1969), found Ach to have an excitatory effect in regions of the ant brain, increasing the firing rate of spontaneously active cells and stimulating silent ones. In one case

neuronal inhibition occurred. The crayfish caudal photoreceptor is also inhibited by Ach (Hermann and Skiles 1969).

The action of Ach in the crustacean nervous system is less well known and at present there is no clear evidence for the presence of cholinergic synapses though Ach, its analogues and anti-cholinesterases have been applied to various ganglionic preparations (see Maynard (1971b)). Florey (1967) has mentioned the possible release of Ach from crab CNS during sensory stimulation. Maynard (1971b) believes it possible that much of the cholinesterase observed in the crustacean CNS may play a protective role, shielding non-synaptic cholinoceptive sites from small changes in level of cholinergic compounds in the milieu. Such cholinergic compounds may be involved in non-synaptic modulation of neuronal activity.

GABA

It now seems highly probable that GABA is the inhibitory transmitter at the arthropod somatic neuromuscular junction. It occurs in the central and peripheral nervous systems of lobsters. In crustacea GABA is present in axons and cell bodies of inhibitory neurones at much higher concentrations than in excitatory neurones. It can be derived from glutamate by the action of glutamate decarboxylase, mimics the effect of stimulating the inhibitory axon and has its action limited to the neuromuscular junction (see Kerkut 1967)for references). GABA is released by inhibitory stimulation at the lobster neuromuscular junction and is inactivated by removal by a specific GABA transport system (see Kravitz 1968).

While the evidence for insects is less complete GABA appears the most likely candidate for the somatic inhibitory neuromuscular transmitter (Kerkut 1967). In the ant brain GABA usually has a clear inhibitory

effect on single units (Steiner and Pieri 1969) and it could possibly also function as a central nervous transmitter.

L-glutamate

The excitatory neuromuscular transmitter in arthropods is most likely glutamate, though some crucial experiments are lacking (Kravitz 1968). In crustaceans L-glutamate occurs in high concentrations in central and motor excitatory axons of the lobster and it mimics the action of the excitatory transmitter (see Strumwasser 1965). It is probably removed from the synaptic region by a specific transport system (Kravitz 1968).

In insects topically-applied L-glutamate excites muscles and nerve stimulation results in the appearance of L-glutamate in the bathing medium (Usherwood et al. 1968). Its depolarizing action is confined to spots believed to coincide with neuromuscular junctions and its main site of action is the muscle fibre membrane itself (Beránek and Miller 1968). L-glutamate may be removed from the synaptic region by diffusion (Usherwood and Machili 1968).

In insects the idea of L-glutamate as a neuromuscular transmitter has to be reconciled with the knowledge that high concentrations of this substance are found in the haemolymph (Usherwood and Machili 1968). However these authors believe that little of the glutamate in the haemolymph is in a 'free' form capable of interfering with synaptic events.

2. Visceral neurotransmitters I

Little is known of visceral neurotransmitters either within ganglia of the stomatogastric system or at gut neuromuscular junctions, and it is possible that the transmission process on the gut differs from that in skeletal musculature (Campbell and Burnstock 1968). Holman and Cook (1970), investigating the pharmacological properties of excitatory

neuromuscular transmission in the hindgut of the cockroach Leucophaea maderae, have even suggested there may be some fundamental physiological differences between the fore- and hindgut.

Cholinesterase activity has been demonstrated in the neuropile of the stomatogastric ganglion of Homarus americanus (Maynard 1971b) but applications of Ach to the ganglion of H. gammarus, while recording intracellularly from ganglionic neurones, had no pronounced effect (M.Dando personal communication).

The effects of various pharmacologically active compounds on the arthropod heart and gut have been mentioned in the introduction and with the exception of L-glutamic acid (glutamate, L-glutamate) in the insect hindgut, there is little indication so far that any of them functions as a visceral transmitter. Holman and Cook (1970) believe that L-glutamate in the cockroach hindgut, satisfies most of the major criteria for recognition of a chemical transmitter proposed by Gerschenfeld (1966, see later), "within the limits afforded by the hindgut nerve muscle preparation used." Thus:-

"(i) Glutamate was found in both the ganglion and nerve leading to the viscera.

(ii) The presence of an effective inactivating mechanism was suggested by the rapid recovery of the hindgut to applied glutamate, even at high concentrations.

(iii) The existence of receptors to glutamate in the effector organ (hindgut) was indicated by the cross-desensitization of glutamate and the natural transmitter, and the blocking of the transmitter receptors by dopamine, an antagonist for glutamate.

(iv) An identity in physiological action was indicated between the

natural transmitter and the applied glutamate at low concentrations."

There is some evidence from the present study and recent papers that biogenic amines may be involved in the visceral transmission process. CA's have been demonstrated, by use of the Falck and Hillarp technique, in both the CNS and so-called autonomic nervous systems of insects (Frontali and Norberg 1966; Plotnikova and Govyrin 1966; Klemm 1968; Björklund et al. 1970) and both CA's (NA and DA) and 5-HT in the CNS of Astacus (Elofsson et al. 1966), and these compounds may play a role in central synaptic transmission (see Treherne 1966; Cook 1967; Kerkut et al. 1969). Monoamines have been demonstrated, also by use of the Falck and Hillarp method, in the visceral nervous system of both insects and crustacea.

Elofsson et al. (1968) showed the presence of monoaminergic fibres in the intestinal nervous system to the hindgut of Astacus astacus. The fibres with specific fluorescence made up only a small part of the intestinal nerve plexus and are believed to contain NA or DA or a mixture rather than A. Osborne and Dando (1970) located monoamines (probably DA but possibly DA and/or NA) in the stomatogastric ganglion of Homarus gammarus, in neuropile and at least some cell bodies. In the present study the presence of DA and/or NA was revealed in the neuropile of the ingluvial ganglion of S. gregaria but specific fluorescence was not detected in the neurone somata. It is possible that small amounts of the amines dispersed in the cytoplasm, might fail to be detected by this method. The ingluvial ganglion of Blabera craniifer has the same distribution of monoamines as S. gregaria (B. Chanussot, personal communication).

Preliminary analysis by paper and thin layer chromatography of extracts of the ingluvial ganglion of S. gregaria has indicated the probable presence of 5-HT (D. Grace, personal communication). DA was

also tentatively identified but further experiments would be required to identify positively both 5-HT and DA.

CA's have been shown to have excitatory effects on the arthropod heart and gut (see introduction) and their presence has now been demonstrated in parts of the visceral nervous system.

3. Ultrastructural localization of biogenic amines.

There has been some controversy over the type of vesicle thought to store biogenic amines (see Fuxe et al. 1966), whether clear or granulated. It is known that CA's strongly reduce osmium tetroxide and that NA-storing granules are made electron-opaque by glutaraldehyde and osmium tetroxide (Bloom and Giarman 1968). The consensus of opinion seemed to be that, at least in central and peripheral monoamine cells of vertebrates the main storage sites of the monoamines were dense-cored vesicles of about 50 nm diameter (see Bloom and Giarman 1968; Hökfelt 1968, 1969; Tranzer and Thoenen 1968b; Watanabe 1970). Reports of mixed populations of small vesicles ('empty' and granulated vesicles of similar size) in what were known or thought to be CA-containing fibres (Tranzer and Thoenen 1967a, 1968b) or of clear vesicles only in areas known to be rich in CA's (Fuxe et al. 1966; Chanussot et al. 1969) seemed to partly insubstantiate this view.

However it is currently thought that the clear and granulated small vesicles represent a homogeneous population differing in degree of amine-filling only (Tranzer and Thoenen 1968b). It has been shown that the fixation technique used is of great importance for the localization of amines in small vesicles. For example Tranzer and Thoenen (1967a) demonstrated that in postganglionic sympathetic nerve terminals, fixation in osmium tetroxide alone showed virtually all the vesicles in the

endings to be empty, giving both cholinergic and adrenergic fibres a similar ultrastructural appearance. Double fixation with glutaraldehyde and osmium tetroxide showed many terminals to contain large numbers of dense-cored vesicles but still within a mixed population. After incubation in a suitable norepinephrine solution and subsequent double fixation virtually all the vesicles in sympathetic terminals were osmiophilic, indicating that the whole vesicle population is at least capable of amine uptake. The results of Tranzer and Thoenen and other workers suggest that the clear or 'empty' vesicles in adrenergic endings are the result of poor preservation of NA during fixation (Tranzer et al. 1969).

By use of a variety of fixation and incubation techniques then, small dense-cored or granulated vesicles may be visualized in nerve terminals containing biogenic amines (Tranzer et al. 1970; Mancini and Frontali 1970).

In nerve terminals containing small granulated or dense-cored vesicles there frequently occur large granular vesicles about 100 nm in diameter, some of which may also contain monoamines (Hökfelt 1968; Tranzer and Thoenen 1968b), but they are not believed to be the main storage site. However in the nonterminal parts of the sympathetic neurone, including ligated axons, the large rather than the small granular vesicles have a distribution that corresponds to that of NA and it has been proposed (see Geffen and Livett 1971) that the large vesicles are precursors of the small type, being synthesized in the neurone soma and transported to the axon terminal where they are transformed to the small type of granulated vesicle. Large granular vesicles also occur in cholinergic nerve terminals but amines have not been located here (Tranzer and Thoenen 1968b).

In the neuropile of the insect ingluvial ganglion, known to contain

CA's, a combination of ultrastructural and histochemical techniques has shown that populations of small clear vesicles (with the occasional granulated one) in at least some fibre terminals are a likely storage site of the amines, and that the amines do not occur in large dense-cored vesicles of other terminals (Chanussot et al. 1969).

Ultrastructural study of the stomatogastric ganglion of Homarus gammarus has shown the existence of two vesicle types in fibre terminals in the neuropile. Similar vesicle types are also present in H. americanus (Maynard 1971a), namely small clear vesicles between 35 and 55 nm in diameter, and larger granulated vesicles between 70 and 110 nm in diameter. The two types occur together in terminals but may be segregated (see Figs. 20 and 21) or partially so. Though monoamines are present in the neuropile of the stomatogastric ganglion it is not known in what type of fibre inclusion they are stored. By analogy with the insect ingluvial ganglion it is probably in the small clear vesicles.

In presumed synaptic regions of the neuropile of the insect ingluvial ganglion (Chanussot et al. 1969) only the small clear vesicles were seen clustered at the presynaptic membrane and never the large dcv (see also Maynard 1971a). In central and peripheral monoamine neurones of vertebrates Hökfelt (1968) found that large granular vesicles were almost always localized relatively far from the synaptic cleft which indicates that they are not directly involved in transmitter release.

4. Visceral neurotransmitters II

The evidence so far on the nature of transmitter substances in the arthropod visceral ganglia is as follows.

(i) While the neurone perikarya of the lobster stomatogastric ganglion contain little or no cholinesterase, suggesting that the neurones are

non-cholinergic, intense cholinesterase activity in the neuropile may mean that cholinergic synapses are formed by extrinsic fibres (Maynard 1971b). There is no evidence for the presence of Ach in either the ingluvial or stomatogastric ganglion.

(ii) Pyloric muscle motor neurones of the stomatogastric ganglion of Panulirus argus excite pyloric muscles but have only inhibitory synapses on to other neurones within the ganglion i.e. they have divergent post-synaptic effects on nerve cells and muscle fibres (Maynard and Atwood 1969). The pyloric muscles are depolarized by glutamate and high concentrations of glutamate inhibit all spontaneous activity in the ganglion, suggesting that glutamate may be a transmitter in the lobster foregut. However, the high concentrations of glutamate required to affect ganglionic activity could rule out a transmitter role (M. Dando, personal communication). The pyloric muscle motor neurones are themselves inhibited by other neurones in the ganglion and unless a cell can both inhibit and be inhibited by the same neurotransmitter, these other neurones must possess a different transmitter.

(iii) There is tentative chromatographic identification of the presence of 5-HT and perhaps DA in the ingluvial ganglion of S. gregaria (D. Grace, personal communication).

(iv) DA and/or NA are present in the neuropile of the ingluvial ganglion of a locust S. gregaria and a cockroach Blabera craniifer, and in the neuropile and some cell bodies of the stomatogastric ganglion of the lobster H. gammarus. It is tentatively proposed that the amines are stored in small clear rather than large granulated vesicles.

Aminergic transmission has been shown to be characteristic of the visceral ganglion of a mollusc (Japha and Wachtel 1969), 5-HT and a

primary CA occur in nerve cells of the pharynx of planarians (Welsh and Williams 1970) and part of the stomatogastric nervous system of the medicinal leech contains monoaminergic (probably a CA) neurones (Rude 1969). Thus it seems that aminergic transmission is common if not universal in the visceral nervous systems of invertebrates, as in vertebrates.

5. Criteria for recognizing a neurotransmitter

Gerschenfeld (1966) has proposed criteria which are necessary for recognizing chemical synapses and neurotransmitters in invertebrates. His essential criteria are listed below:-

(i) presence and storage of the substance in bound form in synaptic vesicles

(ii) presence of a synthesizing enzyme for the substance in synaptic vesicles

(iii) presence of an inactivating enzyme of the substance in the synaptic region, or the possibility of diffusion or refixation to storage

(iv) collection of the substance after nerve stimulation (may be replaced by (i) and (ii))

(v) existence of receptors to the substance in the post-synaptic cell

(vi) identity of physiological action of the substance and the transmitter.

It is therefore clear that with the exception of L-glutamate in the cockroach hindgut (Holman and Cook 1970), none of the known substances which may be involved in arthropod visceral transmission (Ach, 5-HT, DA, NA) come near to satisfying more than one of these criteria and extensive research of a biochemical,histochemical and physiological nature is needed on the visceral nervous system.

6. Origin of the vesicle populations in arthropod visceral ganglia.

Small clear synaptic-type vesicles

It is assumed that at least some of these vesicles store CA's. There have been numerous suggestions for the site of synthesis of synaptic vesicles, which include formation from neurotubules, from mitochondria, by micropinocytosis (see Bunt 1969; Vollrath 1969 for references). However in some neurones known to contain amines, the amines have been found throughout the cell body and thus it seems likely that the vesicles or their components are synthesized in the soma, by ribosomes and at the Golgi apparatus, and carried to the terminals by axoplasmic flow (see Hökfelt 1969; Carlsson 1969; Geffen and Livett 1971).

In electron micrographs of the two arthropod ganglia investigated, many small vesicles are associated with the Golgi bodies, but some of these may represent tubular elements in transverse section. Moreover Hökfelt (1969) points out that since vesicles in the Golgi region may be in a state of growth it is not possible to state definitely that this is where the small granular or agranular vesicles are synthesized. Employment of the argentaffin reaction (see Chanussot et al. 1969) or Wood's technique (Wood 1966) might indicate whether any of the vesicles in the Golgi body contain amines, if the Golgi bodies could be easily demonstrated under these conditions.

The source of the CA's which are present in the neuropile of the insect ingluvial ganglion is unknown. It could be intrinsic neurones or even sensory neurones if these synapse within the ganglion, or neurones of the more anterior visceral ganglia or the CNS.

In Homarus at least some of the neurones of the stomatogastric ganglion have CA's in their cytoplasm (Fig. 22B) and these neurones may or may not be

the sole source of the CA's demonstrated in the neuropile.

Large dense-cored or granular vesicles

In the ingluvial ganglion the large dcv resemble neurosecretory granules described in the nervous systems of a wide variety of animal species, and which occur ubiquitously in the neuropile of insect ganglia (Maddrell 1967). In neuropile fibres the large dcv are generally accompanied by small clear vesicles, with either type predominating. While the majority of the dcv in the neuropile probably represent neurosecretory granules, some of them may correspond to the dcv found in certain aminergic and cholinergic nerve endings (see earlier), and which in aminergic fibres may store CA's.

It is highly likely that more than one and perhaps several types of neurosecretory product are to be found in the neuropile of the ingluvial ganglion. The NSM appears to accumulate in the neuropile (Chanussot et al. 1969) but its origin is unknown. Some at least could derive from intrinsic neurones of the ganglion since dcv resembling those of the neuropile were found to be associated with Golgi bodies. Some or all of the NSM could originate in neurosecretory cells of the brain or corpora cardiaca since fibres from both run in the outer oesophageal nerves, and small numbers of dcv have been seen in a few fibres of these nerves. Strong (1966), using conventional stains for NSM, was able to demonstrate neurosecretory fibres in the outer oesophageal nerves of Locusta migratoria but was unable to demonstrate NSM in the ingluvial ganglion.

Dcv have been found in nerve endings on the midgut of insects (Smith 1968; B.Chanussot 1969, personal communication) and the source of this material could be cells of the ingluvial ganglion or more anterior stomatogastric ganglia, or perhaps more likely, neurosecretory cells of the brain or corpora cardiaca. It is not possible to tell what proportion, if

any, of the NSM in the ingluvial ganglion neuropile, is in through fibres as opposed to fibre terminals.

In the neuropile of the lobster stomatogastric ganglion fibres containing dcv generally also contain small clear vesicles, in varying numbers. As in insects some of the dcv may represent the type of large granulated vesicle present in some aminergic and cholinergic endings, but the large numbers of dcv in some fibres may indicate that they store NSM. Similar dcv are also seen in some fibres of the stomatogastric nerve. The source of dcv in both nsgs and the ganglion could be cells of the more anterior stomatogastric ganglia or the CNS. While dcv were not found to be associated with the Golgi bodies of the stomatogastric ganglionic neurones of Homarus gammarus, this may only have been the result of poor preservation, for Maynard (1971a) has described dcv of slightly smaller size range than those of the neuropile, associated with the Golgi bodies of these cells in Homarus americanus. She also found them to occur infrequently in the neuronal cytoplasm. The neurones of the stomatogastric ganglion therefore may produce some of the dcv observed in electron micrographs of the neuropile.

7. Neurotransmitters and NSM - release and possible effects on the gut

When considering the release of neurohormonal (see Welsh 1961 for terminology) agents from fibre terminals, it appears that for both neurotransmitters and NSM, a mixed population of vesicles is involved. While the function of large dcv in aminergic and cholinergic endings is not known for certain, it seems that in the former but not the latter, these vesicles may store an amine as well as the small clear or granulated vesicles (Tranzer et al. 1969). In neurosecretory endings the role of the small clear synaptic-type vesicles remains to be elucidated. Neurosecretory axons are known to be able to conduct slow action potentials and the small

clear vesicles may perhaps act in a manner similar to synaptic vesicles by affecting the membrane of the cell innervated, where there is direct innervation of the target organ rather than release of NSM in a neurohaemal structure (however see Scharrer 1968). They might also function to alter the permeability of the terminal membrane in the neurosecretory cell, to facilitate release of hormone.

It has further been suggested that the small clear vesicles may be the result of fragmentation of the large dcv (Scharrer 1968), that they may arise from pinocytotic invaginations or neurotubuli (Vollrath 1969) or that they may influence permeability of the membranes of the granulated vesicles to allow diffusion of their contents (see Bern and Hagadorn 1965).

In both the arthropod ganglia examined in this study, the dcv had a variable ultrastructural appearance. A limiting membrane was sometimes apparent, sometimes not, and in some instances was broken. The fragmentation may be a sign of the initial step in release of the NSM or it could be the result of poor preservation of the vesicles.

The variable separation between the vesicle membranes and the dense-cores of the dcv may indicate the degree of filling or emptiness of the vesicles. The wide variation in size of the dcv may indicate the state of growth or depletion, but there may be no critical size for the vesicles. The variation in intensity of staining of the vesicle cores may indicate differences in concentration or change in nature of their contents. In some of the dcv (particularly in H. gammarus) the dense core itself appears granular, as if it is subdivided.

Available evidence so far indicates that exocytosis (fusion of the vesicle membrane with the limiting membrane, followed by extrusion of vesicular contents) is the most likely method of release for NSM (Normann

1965; 1968; Weitzman 1969; Shivers 1969). Other suggested means of release include diffusion from the neurosecretory granule and through the limiting membrane, fracture of the granule and diffusion, or fragmentation to smaller vesicles (presumably followed by diffusion or exocytosis).

It is probable that, even if some of the dcv (NSM?)-containing neuropile fibres of the ingluvial and stomatogastric ganglia are through fibres and proceed to the gut wall, many others represent terminals which release their product within the ganglia. Whether neurotransmitter, or NSM the product could affect fibre conduction and synaptic events within the neuropile or by diffusion in extracellular spaces or extracellular material could affect the neurone somata of the ganglia. Zones called "synaptoid zones" (Scharrer 1968) where neurosecretory fibres apparently synapse onto stroma or glial cells may be one site of release of NSM. Since such zones have been observed in the ingluvial ganglion of Blabera craniifer (Chanussot et al. 1969) it is possible that this ganglion may be a neurohaemal organ. By diffusion through stroma or glial tissue NSM could be released from the ganglion into the haemolymph bathing the gut (or in decapods into the anterior aorta) where it might affect nerve endings or muscle membranes. In insects the control of midgut enzyme production is believed to be hormonal (see Langley 1967) and hormones may also be involved in control of midgut movements (Campbell and Burnstock 1968) but whether these hormones are blood-borne or carried in neurosecretory fibres to the gut is not known.

While neurohormonal agents released within the ingluvial and stomatogastric ganglia may have only an intra-ganglionic action, they may also have an extra-ganglionic action on the gut and particularly in decapods by entering the circulatory system, an effect on other organs such as the heart.

Conversely the ganglia may be easily affected by agents circulating in the blood and released elsewhere. The discovery by Cooke and Goldstone (1970) of a CA-containing cell in the commissural ganglion which has terminals in the pericardial organs, is of interest in this connection. It is possible that sensory input from the gut to the commissural ganglion during feeding could influence the activity of the CA-containing cell, perhaps initiating release of CA in the P.O's. The CA in turn would then enter the heart and be carried forward to the anterior aorta, where it could act upon the stomatogastric ganglion possibly influencing its output and thence foregut activity.

A lot of the discussion is not directly related to LV work. There is also considerable repetition with intro.

V. SUGGESTIONS FOR FURTHER WORK

1. Biogenic amines have been located in both the insect ingluvial ganglion and the crustacean stomatogastric ganglion. The source of the amines is not known in insects and though in the lobster (H. gammarus) the amines are present in some ganglionic cell bodies, these may not be the only source of amines present in the neuropile. To determine the source of ganglionic amines the Falck and Hillarp technique could be used extensively on the SNS, on whole mounts of ganglia and nerves as well as on sections, and on nerves which have been ligatured. This might show decisively whether the insect ingluvial neurones themselves produce amines, and if not, whether they reach the ganglion from an anterior or posterior direction, from the CNS or from more anterior parts of the SNS. In the lobster stomatogastric ganglion, containing a small number of cells, it might be possible to identify neurones showing specific fluorescence by their position in the ganglion, and then perhaps record from known aminergic cells.

The Falck and Hillarp technique could also be used to examine the gut musculature and determine whether amines are present in any of the motor terminals. Nerve endings containing dense-cored vesicles have been described in the arthropod gut but are generally considered to be neurosecretory.

2. An extraction and analysis procedure such as that used by Holman and Cook (1970) on the gut of Leucophaea maderae could be employed to determine what pharmacologically active substances are present in the foregut musculature, and ganglia and nerves of the SNS, which might have a transmitter role there. The effects, if any, of such substances on the isolated gut could be compared with the effects of nervous stimulation.

3. Hildebrandt and co-workers (Hildebrandt 1971) have developed a sensitive method for screening nerve cells for transmitter substances,

which can be used on individual cells such as those of the lobster MRO. Such a method could be used on small groups of sensory cells (e.g. those in the lobster posterior stomach nerve, or those on the anterior midgut of locusts) or on ganglionic extracts. Isolated nerve cells or ganglia are incubated in labelled precursors of the known transmitter substances, and after extraction and separation by high voltage paper electrophoresis the components are examined for the presence of labelled transmitter. Because a cell can synthesize a particular transmitter however does not prove that it is the natural transmitter of that cell and other tests must be carried out. These would include iontophoretic injection of transmitter onto the post-synaptic cell, while recording intracellularly, determining whether the effects of transmitter application mimicked those of nerve stimulation, and perhaps detecting transmitter release during nerve stimulation.

4. Certain aspects of arthropod gut function such as salt and water balance, enzyme production and possibly in some instances, movement, may be under hormonal control. While the hormones may reach the gut in the haemolymph, having been liberated from neurohaemal organs such as the insect corpora cardiaca, or the crustacean sinus gland, they could also be released locally from neurosecretory endings or from ganglia functioning as neurohaemal organs. "Synaptoid" areas (see Scharrer 1968), believed to be release sites for NSM, have been described in the cockroach ingluvial ganglion (Chanussot et al. 1969) and it is possible that ganglia of the SNS could function as neurohaemal organs. Incubation of a ganglion showing "synaptoid" areas, in a solution of labelled biogenic amine (or other putative transmitter) or precursor, followed by use of electron microscopic and autoradiographic techniques might indicate whether these areas in the ganglion investigated are release sites for transmitter or for NSM. If the

latter, the sites would be unlabelled. Such a combination of techniques might also show whether biogenic amines or other transmitters are associated with morphological synapses within the ganglion.

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