SURGICAL INTERFERENCE WITH THE ANTERIOR STOMATOGASTRIC NERVOUS SYSTEM AND ITS EFFECT UPON GROWTH AND MOULTING IN LOCUSTA MIGRATORIA MIGRATORIOIDES R. & F.

by

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Thesis submitted to the University of Nottingham for the degree of Doctor of Philosophy

May, 1973.

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(N.B. In the text Figs. 14-16, Plates 48-53 and Tables 11-20 have been deleted.)

CHAPTER I

INTRODUCTION

At each instar the visible form of the insect body is defined by the cuticle. In the softer parts of the integument the cuticle can unfold and stretch, but in the more inflexible regions, such as the head and appendages, growth cannot occur unless the cuticle is shed. Thus moulting is primarily a mechanism of growth, conditioned by the properties of the cuticle, and serves to allow increase in size and change of form (WIGGLESWORTH, 1965, 1970). Growth implies the production of materials, and in particular proteins, for the formation of the animal (CLARKE, 1965), and is seen to be a continuous process during each instar with only slight discontinuities at ecdysis caused by the loss of the shed cuticle (HIGHNAM and HILL, 1969). The protein requirements for growth are met by the normal food intake and, as CLARKE points out, the link between food intake and growth is of fundamental importance since through it are initiated those physiological mechanisms which will synthesize the nutrients of the food into the specific proteins of the insect's body. The stomatogastric nervous system and the neuroendocrine system constitute this link.

The foregut in insects is innervated by the stomatogastric nervous system (ORLOV, 1924; WILLEY, 1961; CLARKE and LANGLEY, 1963, b, c; DANDO et al., 1968) which is present, in some form or another, in all known insect species (review by CAZAL, 1948). As ROOME (1968) has indicated, the anatomy and function of the stomatogastric nervous system varies according to the mode of feeding of the species concerned. Thus it is generally reduced in liquid feeding insects, where foregut movement is mainly myogenic (JONES, 1960; KNIGHT, 1962), but is more in evidence in insects feeding on solid food stuffs, where movement of the foregut is both myogenic and neurogenic (GRENVILLE, 1962; ROOME, 1968; COOK et al., 1969; MOHL, 1972).

Surprisingly little research has been conducted on the nervous control of foregut movement. In Schistocerca gregaria (CLARKE and GRENVILLE, 1960; GRENVILLE, 1962) movements of the foregut cease altogether after severance of the nerves running from the ingluvial ganglia to the gut. Since the cutting of the outer oesophageal nerves has no effect upon foregut movement these authors conclude that the ingluvial ganglia are autonomous in their effects. According to CLARKE and GRENVILLE the ingluvial ganglia control the contractions of the posterior crop and gizzard, while the hypocerebral ganglion acts by influencing the rate of relaxation of the foregut musculature. The brain and suboesophageal ganglion do not appear to be implicated in the control of foregut movement. Certain regions of the gut, and in particular the oesophagus, display pronounced myogenic activity. ROOME (1968) studied the role of the

stomatogastric nervous system in the control of foregut movement in Locusta migratoria and his results parallel those of the above authors. In addition he found that foregut activity was unaffected by the removal of the frontal ganglion. In Acheta domesticus (MOHL, 1972) the neural mechanism co-ordinating foregut movement is located in the oesophageal nerves, with the hypocerebral and ingluvial ganglia playing merely a stabilizing role.

The involvement of the stomatogastric nervous system in the control of feeding behaviour has been little studied. The results of some ten years' research into the regulation of feeding in the blowfly, Phormia regina, are presented in two reviews (DETHIER, 1969; GELPERIN, 1971). Briefly, food intake in this insect is regulated by the interplay of varying excitatory input from external chemoreceptors and fluctuating feedback from internal mechanoreceptors (stretch receptors) located in the gut and body wall. The stretch receptors are stimulated by the filling of the foregut and their activity, upon arrival at the brain, inhibits the input from the external receptors, resulting in increased taste threshold and consequent cessation of feeding. Information from the foregut receptors is passed to the brain by way of the recurrent nerve. The cutting of this nerve anterior to the stretch receptors interrupts the inhibitory input to the brain and induces hyperphagia. Meal size and the osmotic pressure of the crop contents and blood influence the rate of crop emptying, this process being independent of nervous or endocrine elements.

The stomatogastric nervous system controls crop emptying in Periplaneta and exerts its effect at the level of the proventricular valve (DAVEY and TREHERNE, 1963). The osmotic pressure of the ingested meal, which influences the rate at which the crop empties (TREHERNE, 1957), is detected by a sense organ in the wall of the pharynx. From here information is passed via nerve 5 to the frontal ganglion. A motor pathway, involving the recurrent nerve, the oesophageal nerve and ganglion, and the ingluvial nerve and ganglion, controls the extent and frequency of opening of the proventricular valve. Crop emptying is inhibited when the above pathway is surgically interrupted. In another species of cockroach, Leucophaea maderae, the osmotic values of the food probably do not affect the rate of crop emptying (ENGELMANN, 1968). More important in this respect is the consistency of the food and the initial size of the meal. ENGELMANN considers that the degree of stretch of the crop by the food, and the consistency of the food, is recorded by the stomatogastric nervous system, which in turn controls opening of the proventricular valve via the ingluvial or proventricular ganglion.

The stomatogastric nervous system also controls crop emptying in various acridids, including Schistocerca gregaria (HIGHNAM et al., 1966; HILL et al., 1966); Gryllus bimaculatus (ROUSSEL, 1966); Locusta migratoria (ROOME, 1968); and Melanoplus differentialis (GILLOTT et al., 1970; DOGRA and EWEN, 1971).

In Locusta the stomatogastric nervous system is linked to the cerebral neuroendocrine system by the frontal connectives and, as STRONG (1966) has revealed, by two pairs of fine nerve branches which run from the nervi corporis cardiaci interni to the hypocerebral ganglion.

The insect endocrine system has four major components: groups of neurosecretory cells in the brain, the corpora cardiaca, the corpora allata and the prothoracic glands. Numerous papers have been published on the anatomy and histology of these structures for a wide range of insect groups; some notable reviews are those by CAZAL (1948), WIGGLESWORTH (1964, 1965, 1970), GABE (1966), NOVAK (1966), HERMAN (1967, JOLY (1968) and HIGHNAM and HILL (1969). Particular attention is paid here to the structure of the endocrine system in Locusta.

The cerebral neurosecretory system in acridids has been reviewed by GIRARDIE (1970). In Locusta the neurosecretory cells in each half of the pars intercerebralis form two groups: a medial group (CLARKE and LANGLEY, 1963d; GIRARDIE and GIRARDIE, 1966, 1967) and a lateral group (RAABE, 1964). The medial group contains three cell types: A, B and C (CLARKE, 1966; GIRARDIE and GIRARDIE, 1966, 1967). The majority of the axons from each median neurosecretory cell group decussate within the brain and emerge as the nervus corporis cardiacum internus (NCC I) to enter the contralateral corpus cardiacum. The remaining axons cross over twice and so enter the ipsilateral corpus cardiacum (HIGHNAM, 1969; HIGHNAM and WEST, 1971). The axons

from each lateral neurosecretory cell group constitute the nervus corporis cardiaci externus (NCC II) and run directly to the ipsilateral corpus cardiacum. A third group of protocerebral neurosecretory cells, which are situated below the median ocellus, have recently been described in Locusta and Schistocerca (GIRARDIE, 1970) and Melanoplus (DOGRA and EWEN, 1970). Many insect species, including Locusta, contain a group of neurosecretory cells in each tritocerebral lobe (RAABE, 1963a). The axons of these cells run to the ipsilateral corpus cardiacum as the nervus corporis cardiacum III (NCC III) (RAABE, 1963b). A fourth pair of nerves, the nervi corporis cardiaci IV (NCC IV), which leave the posterior face of the deuterocerebrum and run to the corpora cardiaca, have been described in Locusta, and a number of other insect species, by BROUSSE-GAURY (1967). The cell bodies of these nerves have not been identified in Locusta, but BROUSSE-GAURY has been able to detect a few "Gomori positive" cells in the deuterocerebrum of Dytiscus marginalis which, she believes, may be the perikarya of the NCC IV.

The corpus cardiacum in Locusta has been variously described, both at the light (NAYAR, 1954; OZBAS, 1957b; CLARKE and LANGLEY, 1963d; CASSIER, 1965) and ultrastructural (CASSIER and FAIN-MAUREL, 1970a, b; CAZAL et al., 1971) levels. It is made up of the bulbous endings of the neurosecretory axons from the brain, carried in the NCC I-IV, but also contains intrinsic glandular cells of its own.

Closely associated with the corpus cardiacum is the corpus allatum, whose structure in Locusta has been described at the light level by OZBAS (1957a), JOLY (1960), ANSTEE

(1968) and CLARKE and ANSTEE (1971), and at the ultrastructural level by JOLY et al. (1967, 1968, 1969). The corpus allatum contains numerous glandular cells as well as axon termini derived from the nervus corporis allatum I (NCA I), and nervus corporis allatum II (NCA II). The NCA I links the corpus allatum to the corpus cardiacum, while the NCA II links it to the sub-oesophageal ganglion (STAAL, 1961). Neurosecretory material has been detected in the NCA I (CASSIER and FAIN-MAUREL, 1970b) and NCA II (CHALAYE, 1965, 1966, 1967).

The prothoracic glands in Locusta are composed of a thin sheet of cells lying at the postero-lateral edge of the head capsule (STRICH-HALBWACHS, 1954). Their ultrastructure in the nymphal form has been described by FAIN-MAUREL and CASSIER (1968). CLARKE and LANGLEY (1963a) could find no trace of nerves to the prothoracic glands in Locusta, but according to CHALAYE (1965, 1966) the glands in this insect are innervated by nerves from the sub-oesophageal ganglion. In Leucophaea (SCHARRER, 1964), Calliphora erythrocephala (NORMANN, 1965) and Tenebrio molitor (ROMER, 1971) the nerves innervating the prothoracic glands contain both neurosecretory and non-neurosecretory axons.

There exist numerous examples of the control of metabolic processes, other than moulting, by the secretions of the insect neuroendocrine system (see recent reviews by JOLY and CAZAL, 1969; WIGGLESWORTH, 1970). As early as 1936, WIGGLESWORTH had clearly demonstrated that the corpora allata were essential for the normal maturation of eggs in

adult Rhodnius prolixus. Shortly afterwards PFEIFFER (1939) showed that ablation of the corpora allata in adult Melanoplus prevented egg development beyond the stage at which yolk deposition normally occurred. Since these early discoveries the relationship between corpus allatum activity and egg development has been confirmed in a number of insects (reviews by JOHANSSON, 1958; ENGELMANN, 1968).

THOMSEN (1952) was the first to show that the median neurosecretory cells of the pars intercerebralis are essential for ovarian development and normal reproduction in Calliphora. Many authors have since demonstrated the importance of the neurosecretory cells for the complete development of the oocytes (HIGHNAM, 1962; MORDUE, 1965; LEA, 1967; WILKENS, 1968).

The protein metabolism of the whole insect is also under neurosecretory control. In Calliphora (THOMSEN and MOLLER, 1959, 1963), Tenebrio (MORDUE, 1967) and Melanoplus (DOGRA and GILLOTT, 1971) the neurosecretory hormone regulates midgut protease synthesis. In Schistocerca the neurosecretory cells of the pars intercerebralis and corpora cardiaca exert a controlling influence over the protein content of the haemolymph and the protein synthetic activity of the fat body (HILL, 1962, 1965; OSBORNE et al., 1968). A number of workers have also implicated the corpora allata in the control of protein synthesis (BODENSTEIN, 1953; L'HELIAS, 1957; ROLLER, 1962; MINKS, 1967).

Neurosecretory factors influence many other body processes including: excretion and water balance (MADDRELL, 1963, 1964; BERRIDGE, 1966; CAZAL and GIRARDIE, 1968;

MORDUE, 1969, 1970), cuticular hardening and darkening (FRAENKEL and HSIAO, 1965), gut contractions (review by DAVEY, 1964; CAZAL, 1969), rate of heart beat (CAMERON, 1953; DAVEY, 1961; MORDUE and GOLDSWORTHY, 1969), blood sugar level (STEELE, 1961, 1963; MORDUE and GOLDSWORTHY, 1969) and pigmentation (RAABE, 1963c; GIRARDIE, 1967).

A natural outcome of the study of insect neuroendocrine function has been the discovery of a variety of stimuli for initiating neurosecretory activity. One of the most important of these is feeding. A single blood meal stimulates the release of neurosecretion in Rhodnius, the stimulus reaching the cerebral neuroendocrine system via the central nervous system (WIGGLESWORTH, 1934; VAN DER KLOOT, 1960, 1961). When starved Schistocerca (HIGHNAM et al., 1966), Locusta (HIGHNAM and WEST, 1971) or Melanoplus (DOGRA and GILLOTT, 1971) are allowed to feed there follows a fairly rapid depletion of the accumulated stainable material from the neurosecretory system. This suggests the involvement of a direct control mechanism, perhaps via chemoreceptors situated on the posterior surface of the labrum (DAVEY, 1961, 1962a, b) or via foregut stretch receptors (CLARKE and LANGLEY, 1963c).

A variety of environmental factors affect neurosecretory activity in insects. The studies of LEES (1964) and WILLIAMS and ADKISSON (1964) strongly suggest that light has a direct effect on the medial neurosecretory cells. Further evidence for this stems from the work of CYMBOROWSKI and DUTKOWSKI (1969, 1970). COOK and MILLIGAN (1972) have shown

that light can affect both the 'resting' and spike potentials of the median neurosecretory cells in Periplaneta, and they suggest that this might cause changes in synthesis and/or secretory activity of these cells. The photo-neuroendocrine pathways influencing the activity of the cerebral neurosecretory centres in cockroaches have been traced by BROUSSE-GAURY (1968a, b, 1969a). Here, fibres of ocellar nerves synapse with neurosecretory perikarya of the cerebral ganglion whose axons comprise the NCC I, II and IV. The neurone cell bodies of the latter nerve also receive sensory input from the antenna (BROUSSE-GAURY, 1968c), while those of the NCC III synapse with the labral nerve (BROUSSE-GAURY, 1969b). CLARKE (1966) has shown that temperature can affect the amount of material present in the corpora cardiaca. Thus, in 6 day-old adult Locusta the amount of neurosecretory 'A' material in the anterior lobes at 15°C is less than at 30°C or 45°C. Widely fluctuating temperature regimes (e.g. 30° $\pm$ 15°C) have a more dramatic effect upon the corpora cardiaca, leaving them completely depleted of neurosecretory material.

Other naturally occurring phenomena that bring about the rapid release of material from the neurosecretory system include copulation (HIGHNAM, 1961, 1962; HIGHNAM and LUSIS, 1962), oviposition (HIGHNAM, 1962), and flying (HIGHNAM and HASKELL, 1964). Neurosecretory release can also be induced by artificial means, as for example by electrical stimulation (HODGSON and GELDIAY, 1959; HIGHNAM, 1961, 1962; SCHARRER and KATER, 1969; NORMANN, 1969), enforced activity (HODGSON and GELDIAY, 1959; HIGHNAM, 1961, 1962) or drastic wounding (HIGHNAM, 1962).

It is not known for certain what causes the discharge of neurosecretory material from its intraneuronal storage sites but there are reasons to suppose that this process is correlated with action potentials conducted by neurosecretory neurones (COOKE, 1967; GOSBEE et al., 1968; COOK and MILLIGAN, 1972). The work of BERLIND and COOKE (1968) indicates that the release of neurosecretory material in invertebrates, like that in vertebrates, is a calcium-dependent process. NORMANN (1965) has suggested that neurosecretory granules can only be discharged when a nerve impulse depolarizes the axon membrane and makes it possible for the granule membrane to fuse with the cell membrane. Granule liberation may be achieved either by exocytosis or by intracellular fragmentation (review by SCHARRER and WEITZMAN, 1970).

The historical aspects of the endocrine control of moulting are well known to insect physiologists. KOPEC (1922) was the first to demonstrate that the brain was the source of a hormone necessary for growth and metamorphosis. Subsequent work in other Lepidoptera by HACHLOW (1931) indicated that a region in the thorax was controlling growth and metamorphosis. Nine years later FUKUDA (1940) proved that the 'prothoracic glands' in larvae and pupae of Bombyx mori were the immediate source of the moulting hormone.

In a series of papers WIGGLESWORTH (1934, 1939, 1940) showed that the neurosecretory cells of the pars intercerebralis produce a hormone which initiates moulting in Rhodnius, and that the corpora allata normally furnish a "metamorphosis

inhibiting hormone", the secretion of which fails in the last nymphal instar thus permitting the development of the adult characters at the final moult.

WILLIAMS (1947) proved that the prothoracic glands in Hyolophora cecropia were stimulated by factors from the neurosecretory cells of the brain to secrete their own hormone which brings about the termination of pupal diapause.

Some thirty years after KOPEC's initial discovery WIGGLESWORTH (1952) brought together the available evidence and showed that the "moulting hormone" he had described in 1934 was in fact a composite factor, consisting of an activation hormone from the brain and a moulting hormone from the prothoracic glands.

Since these early studies there have appeared many reviews on the regulation of growth and moulting in insects by the interaction of factors from the brain, corpora allata and prothoracic glands (WIGGLESWORTH, 1957, 1964, 1970; LEES, 1955; VAN DER KLOOT, 1960; DE WILDE, 1962; KARLSON, 1963; SCHNEIDERMAN and GILBERT, 1964; HIGHNAM, 1967; JOLY, 1968).

The manner in which the brain hormone influences the prothoracic glands has been little studied. In Rhodnius (WIGGLESWORTH, 1934) the prothoracic glands must be exposed to the brain hormone for a certain "critical period" in order that the moulting cycle might proceed to completion. This is also the case in Calpodes ethlius (LOCKE, 1970) and in the adult apterygote, Thermobia domestica (WATSON, 1964). Extirpation of the pars intercerebralis (GIRARDIE, 1964) or the prothoracic glands (JOLY et al., 1956; STRICH-HALBWACHS,

1959) in Locusta nymphs before the critical period leads to a permanent arrest of moulting.

In Rhodnius the brain hormone causes the cells of the prothoracic glands to undergo a conspicuous cycle of secretory activity, with the nuclei increasing greatly in size during the critical period. OBERLANDER et al. (1965) have shown autoradiographically that within 12 hr of exposure to brain hormone the prothoracic gland cells in the pupa of Antheraea polyphemus are engaged in active nuclear RNA synthesis. This is followed by the appearance of cytoplasmic RNA and by protein synthesis, events which are taken to represent the synthesis of enzymes necessary for the production of ecdysone (SCHNEIDERMAN and GILBERT, 1964). In Periplaneta the cells of the prothoracic glands are stimulated to synthesize nuclear RNA within 8 hr of brain hormone application (GERSCH and STURZEBECHER, 1970). One of the responses that the prothoracic glands in Locusta make upon the release of the brain hormone is an increase in cell number (CLARKE and LANGLEY, 1963a); another is an increase in nuclear size by some but not all of the gland cells (CARLISLE and ELLIS, 1968).

The moulting hormone exerts its effect upon the epidermal cells by setting in motion an orderly sequence of events which occur synchronously in all parts of the body. This sequence includes apolysis (separation of the cuticle from the underlying epidermis), cell enlargement and associated protein synthesis, secretion of the new cuticle, and resorption of the old cuticle. In the integument of Locusta (JOLY, 1955) a wave of mitoses in the epidermal cells is an integral part of the moulting process; but in Rhodnius (WIGGLESWORTH, 1940, 1963) moulting may occur with almost no mitoses at all.

KARLSON (1956) isolated a pure, chemically defined hormone from pupae of Bombyx and named it ecdysone; but although this hormone initiates the changes in the epidermis normally associated with moulting, its presence in the prothoracic glands has still to be demonstrated. ELLIS et al. (1972) have recently shown that the prothoracic glands in Schistocerca contain two substances which cause apolysis and early stages of moulting to take place, but do not contain a third substance, 20-hydroxyecdysone, which is necessary for the formation of the new cuticle (MORGAN and WOODBRIDGE, 1971). It has been suggested that the prothoracic glands produce another hormone or hormones, which trigger(s) the formation of 20-hydroxyecdysone in another part of the body, possibly the oenocytes of the abdomen (WEIR, 1970; LOCKE, 1969; ROMER, 1971).

As well as being activated by the brain hormone and possibly directly influenced by nervous elements, the prothoracic glands can also be activated by ecdysone itself, a mechanism which perhaps ensures the synchronous secretion of each of the paired glands (WILLIAMS, 1952; WIGGLESWORTH, 1964). Furthermore, the prothoracic glands in certain Lepidoptera can be activated by the corpora allata (GILBERT and SCHNEIDERMAN, 1959; ICHIKAWA and NISHIITSUTSUJI-OWA, 1959; WILLIAMS, 1959; SCHNEIDERMAN and GILBERT, 1964). There is, however, a strong possibility that this effect is a pharmacological one (HERMAN, 1968).

It is clear, then, that the release of the brain hormone is the initial step in a sequence of events that lead up to a moult. But despite the importance of this step, there exist

only a few examples of the stimuli responsible for initiating the release of the brain hormone, and most of these are rather specialized.

On severing the ventral nerve cord in the prothorax of a newly fed nymph of Rhodnius, WIGGLESWORTH (1934) found that the animal would not moult although its endocrine system was still fully intact. He concluded that the stimulus for initiating the moulting cycle was the stretching of the abdomen rather than the animal's state of nutrition, since Rhodnius could live for long periods if fed on small blood meals. VAN DER KLOOT (1961) has since demonstrated the existence of stretch receptors in the abdomen of this insect which, when stimulated, cause impulses to appear in the nervi corporis cardiaci, an effect also produced following stimulation of the ventral nerve cord.

KEMPER (1931) also concluded that distension of the abdomen was important in the initiation of moulting in the bed-bug, Cimex lectularis.

Since in their natural habitat these two insects only occasionally obtain a full blood meal, this method of initiating the moulting cycle must be regarded as a special adaptation.

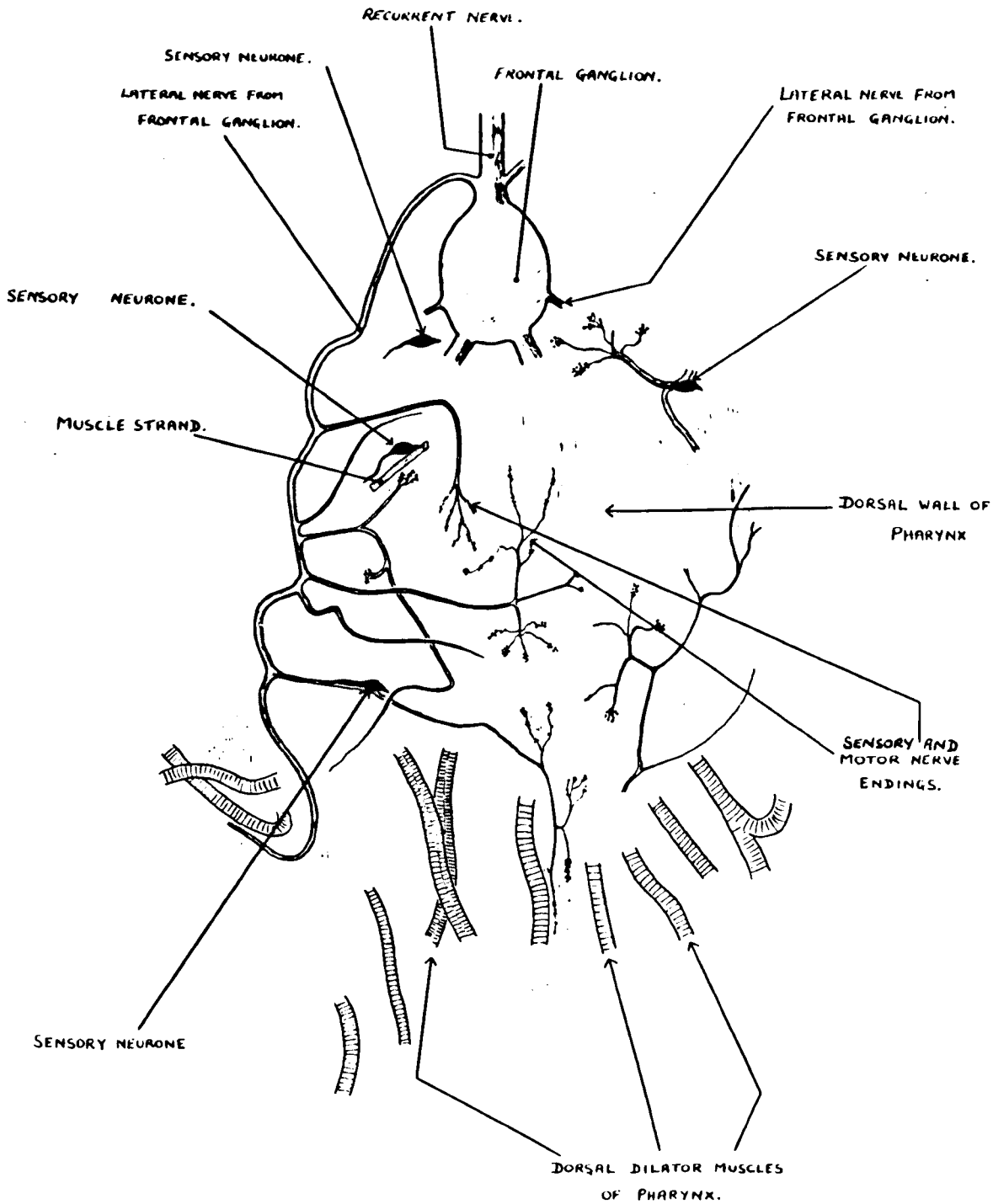
Other known mechanisms for initiating moulting are also rather exceptional. For example, the squash fly, Zeugoducus depressus, which as a larva lives in the cavity fresh fruit, does not undergo pupation until the concentration of carbon dioxide in its environment has fallen from about 6% to 1%, usually after six months storage (TAKAOKA, 1960).

In insects that feed more or less continuously throughout their lives the moult-inducing mechanism has received scant attention. One notable exception is the work of CLARKE and LANGLEY (1963a, b, c, d). During an investigation into the factors which initiate growth and moulting in Locusta, these authors discovered a number of operations which, when performed before a certain critical period, not only prevented moulting, but also arrested growth (LANGLEY, 1962). Since the work to be described in this thesis is, in part, a direct continuation of this investigation, a resumé of the more important results and conclusions is now given.

Methylene blue preparations of the anterior region of the head revealed that, in addition to the three main nerves which leave the frontal ganglion (the two frontal connectives and the recurrent nerve), there were a number of finer nerves associated with the sensory neurones which lie close to the surface of the pharynx (LANGLEY, 1962; CLARKE and LANGLEY, 1962, 1963b, c) (Diagram I). These sensory neurones resembled those described by other workers in the Orthoptera (ZAWARZIN, 1916), Coleoptera (ORLOV, 1924), and Hymenoptera and Lepidoptera (FINLAYSON and LOWENSTEIN, 1958). It thus seemed that the frontal ganglion was well equipped to receive sensory impulses arising from distension and relaxation of the foregut (LANGLEY, 1962).

Following the removal of the frontal ganglion, the cutting of both frontal connectives, or the separation of the frontal ganglion from the surface of the foregut (which involved severance of the anterior and posterior pharyngeal nerves), there was an immediate and irreversible cessation of

DIAGRAM I



INNERVATION OF THE DORSAL PHARYNGEAL WALL FROM THE FRONTAL GANGLION.

(By kind permission of Dr. P. Langley.)

growth, as indicated by body weight. On the other hand, the cutting of the recurrent nerve or only one frontal connective had little or no effect on growth and moulting in the animal (CLARKE and LANGLEY, 1963b, c).

Evidence for the endocrine control of protein metabolism in insects has been indicated above, and it was thought by CLARKE and LANGLEY (1963d) that these "growth arresting operations" might be mediating their effect via the neurosecretory system of the animal on protein metabolism. To test this view they compared the neuroendocrine system of a normal animal with that of an animal subjected to a growth arresting operation (frontal ganglionectomy). The neuroendocrine system in normal animals, examined from the beginning of the third instar until the middle of the fifth instar, revealed no histological signs of a cycle of secretion which could be correlated with the progress of the growth and moulting cycle. The secretory cells of the pars intercerebralis presented a constant picture as too did the corpora cardiaca. Only on rare occasions, and in minute quantities, could neurosecretory material be detected in the NCC I. The neuroendocrine system in frontal ganglionectomised third instar nymphs resembled that in the normal insect for some time after the operation. However, by about 200 hr after the operation the axons of the NCC I had become loaded with neurosecretory material where they emerge from the brain. The corpora cardiaca at this time were abnormal, appearing shrunken and with little sign of cytological detail.

As a result of their studies CLARKE and LANGLEY (1963d) have proposed the following hypothesis of the control of

growth and moulting in locusts:

Throughout a single instar the insect feeds, digests, absorbs and assimilates its food and increases its body tissue content. At this time the foregut is continually being exercised with the passage of food. These distortions of the foregut stimulate the stretch receptors situated on its surface to send nervous impulses to the brain via the posterior pharyngeal nerves, frontal ganglion and frontal connectives, and to the corpora cardiaca via the recurrent nerve and hypocerebral ganglion. On arrival at the brain the nervous impulses stimulate the medial neurosecretory cells of the pars intercerebralis to synthesize, transport, and release their secretions into the blood. During the instar feeding period these secretions exert a controlling influence over food metabolism. Towards the end of the instar the insect ceases to feed prior to moulting (CLARKE, 1956). However, movements of the foregut do not cease, and may even be accentuated, firstly in the process of emptying the gut of food, and secondly in the swallowing of air, which is a necessary preliminary to the shedding of the old cuticle. Therefore, information continues to pass to the brain and more hormone is synthesised and released. Since this hormone is no longer required for the metabolism of food material, its titre in the blood is raised to a critical point at which the prothoracic glands are triggered into activity. After ecdysis, feeding is resumed and the hormone titre falls as the brain hormone becomes involved once more in the control of food metabolism. CLARKE and LANGLEY consider that this

hypothesis can be applied to all continuously feeding insects whose development is not arrested by other phenomena during the course of their life cycles.

The hypothesis is strengthened by the work of CLARKE and GILLOTT (1967a) who demonstrated a marked reduction in protein synthesis, as measured by the incorporation of ^{14}C-glycine into protein, after the removal of the frontal ganglion from third instar Locusta nymphs. In addition, it was shown that the injection of corpora cardiaca extract into frontal ganglionectomised locusts led to a resumption and continuation of growth, indicating that the effects of this operation are mediated through this endocrine organ. At the cellular level CLARKE and GILLOTT (1967b) found that frontal ganglionectomy adversely affected the ability of many tissues to synthesis RNA in the nucleus. They suggest that the two best known effects of the brain hormone, protein synthesis and activation of the prothoracic gland, can be explained by attributing a common function to the brain hormone, namely that of promoting the synthesis of messenger RNA within the cell.

CLARKE and ANSTEE (1971b) report an accumulation of neurosecretory material in the NCC I of frontal ganglionectomised fifth instar Locusta nymphs within 72 hr of the operation, over 100 hr earlier than in the operated third instar nymphs of CLARKE and LANGLEY. At the ultrastructural level, the manufacture of neurosecretory granules by the Golgi bodies appeared to be unaffected by the operation, a result strengthening the view of CLARKE and GILLOTT (1967a) that frontal ganglionectomy acts at the level of neurosecretory

release rather than at the level of neurosecretory synthesis. The cells of the midgut and fat body were found, when examined electron microscopically, to exhibit typical signs of reduced protein synthesis (CLARKE and ANSTEE, 1971a). This is attributed to lack of brain hormone, and possibly also lack of juvenile hormone, since the corpora allata in operated nymphs appear shrunken and histological inactive (CLARKE and ANSTEE, 1971b).

Since the publication of CLARKE and LANGLEY's original series of papers in 1963, a number of investigators have found that frontal ganglionectomy does not always block growth in every operated animal. Thus, while the majority of the frontal ganglionectomised Locusta and Schistocerca nymphs of ROOME (1968) maintained a constant weight after the operation, a few showed fairly significant weight increases and displayed well developed fat bodies at autopsy. According to ROUSSEL (1966) removal of the frontal ganglion from young and mature adults of Necrophorus vespillo has no adverse effect upon growth, the operated animals continuing normal alimentation and increasing their body weight. Protein digestion and syntheis does not stop when the frontal ganglion is removed from adult female Melanoplus (DOGRA and EWEN, 1971), the operated animals laying eggs at a rate equal to about 60% of that for normal mated females. Neither is protein syntheis blocked in frontal ganglionectomised Periplaneta nymphs (PENZLIN, 1971), since these animals continue to regenerate legs in the normal manner after the operation.

Two operations are central to the hypothesis of CLARKE and LANGLEY: the severance of both frontal connectives, and the separation of the frontal ganglion from the surface of the foregut (involving severance of the pharyngeal nerves). Both operations were attended by an early high mortality and the growth curves presented by CLARKE and LANGLEY (1963c) are representative, in each case, of a few animals only. In view of the importance of these two operations, and taking into consideration the above mentioned discrepancies associated with the operation of frontal ganglionectomy, it was considered necessary to repeat all three operations and to check their effect on the growth and moulting cycle in Locusta migratoria migratorioides. In addition the anatomy and fine structure of certain components of the stomatogastric nervous system is investigated, and the results obtained are presented in this thesis.

CHAPTER II

MATERIAL AND METHODS

A. Maintenance of the stock animals

The work to be described in this thesis has been carried out on Locusta migratoria migratorioides R. and F., phase gregaria, stocks of which were originally supplied by the Anti-Locust Research Centre.

The locusts were reared in a centrally heated insectary at a temperature of 28 ± 0.5°C and a relative humidity of 70 ± 5%. Air was circulated through the room by two large electric fans attached to the ceiling; in addition slight continuous air exchange was permitted by two small ventilators. General illumination was provided by five 80 watt fluorescent strip lights.

Populations of Locusta were housed in glass-fronted metal cages of the type recommended by the Anti-Locust Research Centre (HUNTER-JONES, 1961). The area containing the locusts measured 43 cm high x 38 cm wide x 38 cm deep. This was separated from the true base of the cage by a false floor

constructed of perforated plated steel which allowed all faeces, except those of adults, to pass through to the space underneath. The space between the false floor and the true base measured 10 cm. Several circular holes, 4 cm in diameter, were cut into the false floor and into them were inserted aluminium tubes filled with a moist silver sand/peat mixture (75%/25% by volume) for the deposition of egg pods by the sexually mature females. The tubes were replaced daily thus making it possible to define to within 24 hr the age of the eggs (LANGLEY, 1962).

The sides and back of the cage were lined with muslin to increase the area over which the locusts could move. Each cage was illuminated by a single 25 watt light bulb which, together with the general illumination of the insectary, was controlled by a time-switch to give a 12 hr light/12 hr dark period each day. Air circulated freely in and out of the cage via a small perforated area in the roof and the open space between the false perforated floor and the true base. Access to the locusts was by a trapdoor in the roof of the cage. High density cultures were maintained to ensure no reversion to the solitary phase (HUNTER-JONES, 1961).

Freshly picked grass of a good quality was administered every day to the nymphal and sexually mature populations of Locusta. Mortality among newly emerged and maturing adults

was greatly reduced by supplying them with grass picked the previous day and therefore containing less moisture than the freshly picked form. Flake bran was used to augment the grass diet, offering as it does protein without associated water. In Schistocerca adults (HILL et al., 1968) the omission of bran from the diet slows down the rate of somatic growth and delays the onset of oocyte development.

Under the rearing conditions described a pre-adult life span of about four weeks was obtained.

B. Maintenance of the experimental animals

(i) Fourth and fifth instars

Fourth and fifth instar nymphs were kept singly in 8 oz screw-cap glass jars (350 ml capacity) with perforated metal lids. The jars contained a floor and perch of filter paper which served to soak up excess moisture from the grass and faecal pellets as well as any haemolymph escaping from the operation wound. The filter papers were replaced at regular intervals during the course of an experiment.

When moulting was imminent (indicated by a drop in weight and softening of the cuticle) fifth instar experimental animals were transferred to plastic containers measuring

13.5 cm high x 10 cm wide x 10 cm deep. The lids were of gauze with a plastic rim. Each box contained a floor and perch of filter paper, the locusts moulting from either the perch or the gauze of the lid.

Fresh grass was supplied daily in amounts only just exceeding that which could be consumed in a day. Bran was not provided. The experimental nymphs were exposed to the general illumination of the insectary only and, as in the stock cages, the relative humidity of their environment was somewhat variable.

No signs of phase change were apparent in locusts kept individually (CLARKE and GILLOTT, 1967 a).

(ii) Adults

Adults were kept separately in glass jars at room temperature for two days after the operation. They were returned to the insectary on the third day and transferred to an empty stock cage where they spent the rest of the experimental period under crowded conditions. Fresh grass and bran were offered daily.

C. Sampling technique

In order to obtain a uniform population some sort of sampling technique is essential (LANGLEY, 1962). The technique employed was similar to that previously described by GILLOTT (1965), ANSTEE (1968) and ROOME (1968). Let us suppose that fifth instar locusts were required, then once the fourth ecdysis was under way all the fifth instars present in the stock cage were removed each day at 9 a.m. and 5 p.m. This continued until sufficient animals for the particular experiment were obtained at any one sample. During the present study only those nymphs moulting between 5 p.m. and 9 a.m. were used. The age range of the sampled population was therefore 8 hr $\pm$ 8 hr.

Since the ecdysial time range of the fifth ecdysis is the longest (LANGLEY, 1962) it was found necessary to extend the sampling period for adults to 24 hr so that sufficient numbers of locusts could be obtained at a single sample. Newly emerged adults were sampled each day at 9 a.m. thus giving the sampled population an age range of 12 hr $\pm$ 12 hr.

Newly moulted deformed nymphs or fledglings were discarded as were excessively light or heavy locusts; acceptable weight ranges were as follows:

Fourth instar	Male	190-250 mg
	Female	210-280 mg
Fifth instar	Male	400-550 mg
	Female	500-650 mg
Adult	Male	900-1250 mg
	Female	1100-1500 mg

Animals sampled for an experiment were randomly divided into two, and very occasionally three, groups:

(a) Operated animals were subjected to various operations, involving nerve severance, performed on the anterior stomatogastric nervous system or occasionally on the central nervous system. The operations were always carried out during the first 30 hr of the instar.

(b) Control animals were treated in an identical manner to the operated group except that the nerves were merely touched and not cut.

(c) Normal or unoperated control animals were only occasionally used. They were sampled, weighed and then immediately returned to insectary conditions.

D. Anaesthesia

Locusts were anaesthetized in an 8 oz screw top jar containing a cotton wool plug soaked in anaesthetic ether (MacFarlan Smith Ltd.). A narrow layer of cotton wool and a few filter papers were placed on top of the ether plug to prevent the locust coming into direct contact with the liquid anaesthetic. Each animal was anaesthetised for three minutes after which time it had become completely immobile.

E. Sterilization

A fairly rigid sterilization procedure was adopted which, when adhered to, led to a high survival rate among the operated animals. Glass jars were sterilized for 30 min in an autoclave at 250°F (15 pounds per square inch pressure) and instruments exposed to ultra-violet light in a dust-proof cabinet for 30 min immediately before use (CLARKE and LANGLEY, 1963 b). The ringer solution (HOYLE, 1955) used during the operations was freshly made up each time, sterilized by membrane filtration and administered from sterilized 1 ml disposable syringes (Gillette Scimitar). The metal lids of the glass jars were also sterilized in ultra-violet light for a minimum period of 30 min.

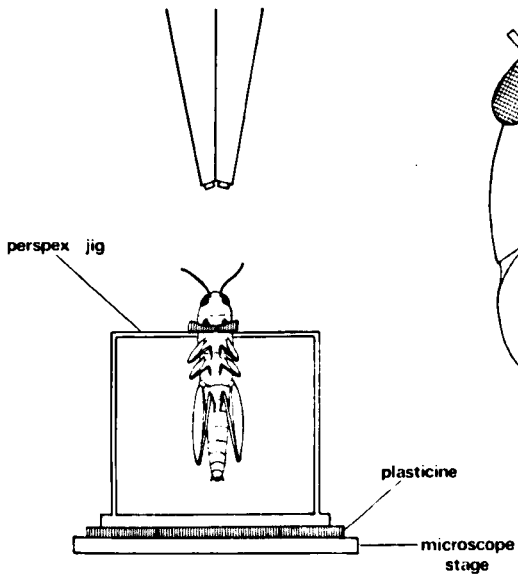
F. Surgical procedure

(i) Frontal approach

For operations in the vicinity of the frontal ganglion the LANGLEY (1962) and CLARKE and LANGLEY (1963 b) method of entry into the head capsule was adopted. Thus the anaesthetized insect was placed in a perspex jig with the frons, previously wiped clean, pointing upwards. The jig was transferred to the stage of a binocular microscope and held in place by plasticine (Diagram IIa). Illumination was provided by two high intensity lamps to which were fitted polaroid heat filters. Cuts 1-3 (Diagram IIb) were made in the frons and the U-shaped flap of cuticle and hypodermis so formed turned down ventrally to reveal three large frontal air sacs. These were carefully removed to expose the frontal ganglion and its associated nerves lying on top of the pharynx (Diagram IIc). Sterile ringer solution was immediately dispensed into the wound; its effect was to cause the frontal ganglion to lift slightly from the surface of the gut, thus facilitating detection of the fine pharyngeal nerves which connect the ganglion to the gut surface.

The frontal connectives, recurrent nerve and pharyngeal nerves were severed in two places and the middle portion removed to minimize the chances of the two cut ends rejoining.

DIAGRAM II



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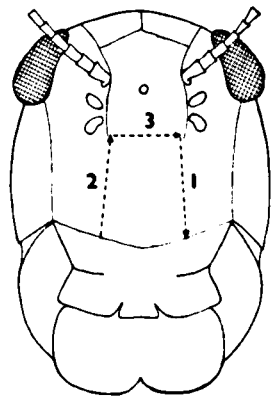

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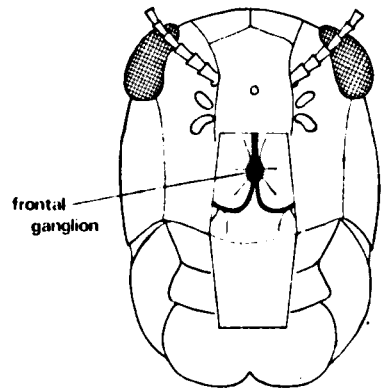

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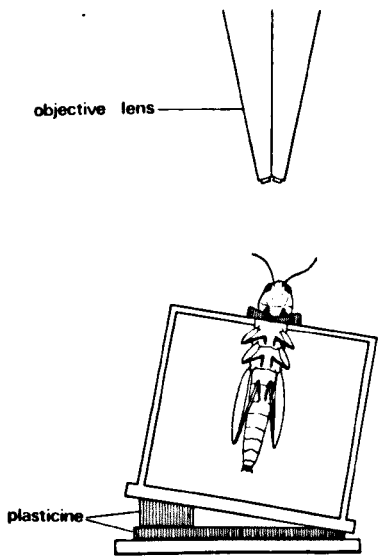

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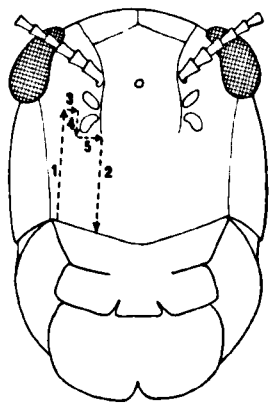

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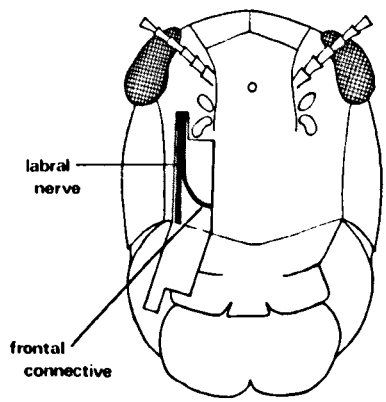


f

Removal of the frontal ganglion was accomplished by carefully severing all its attendant nerves and then simply picking it up from the surrounding fluid. Upon completion of the operation the flap was replaced into position and the wound left to heal without the addition of any sealing materials.

(ii) Front-lateral approach

A fronto-lateral method of approach into the head capsule was adopted during the performance of operations involving severance of the labral nerve or that part of the frontal connective which runs alongside it. The insect was fixed into the perspex jig, transferred to the microscope stage and the fronto-lateral region of the frons brought into the field of focus by propping up one end of the jig with a piece of plasticine (Diagram IId). Cuts 1-5 (Diagram IIe) were then made, the light brown oval patch of cuticle, which marks the point of origin of various head muscles, being carefully avoided. The flap of cuticle and hypodermis was turned down ventrally and sterile ringer solution immediately added to the preparation. The labral nerve and frontal connective could then be seen running close and parallel to one another (Diagram IIf). They can be distinguished on three counts: (1) the frontal connective lies inside the labral nerve; (2) it is thinner than the labral nerve; (3) a large trachea

frequently runs along the length of the frontal connective but never along the labral nerve. The appropriate nerve(s) was severed in two places, the middle portion removed and the flap of cuticle replaced.

The same cuts were made in controls as in operated animals. Sterile ringer solution was added to the preparation after the flap of cuticle had been turned back. The air sacs were removed (in the frontal approach), the appropriate nerve(s) or ganglion touched with fine forceps and the flap then replaced.

G. Post-operative treatment

Each operated animal was placed in a screw-cap glass jar (described previously) which in turn was transferred to a dust-proof cabinet at room temperature (about 21°C). This kept the animals relatively inactive until the wound had had time to heal (CLARKE and LANGLEY, 1963 b). The locusts remained unfed on the day of the operation (Day 1) but received grass the following day (Day 2). On Day 3, 48 hr after the operation, the animals were returned to the insectary and providing the procedures outlined above had been strictly adhered to it was possible to achieve 100% recovery from the operation.

H. Post-mortem examinations

Every insect that died or was sacrificed during the course of an experiment was subjected to a post-mortem examination. This was made to ensure that the appropriate nerves had been cut successfully (or ganglion removed) and that the cut ends had not rejoined. In cases of reconnection, or where some doubt existed, then the insect was excluded from the experimental data. Notes were made on the state of the animal at the time of death, e.g. formation of a second cuticle, gut contents, etc.

I. Measurement of growth

Changes in wet weight offer little indication of growth rate since they take into account neither variation in the amount of food in the gut nor variation in body water content from one individual to another. However, when information on day to day changes in the growth rate of an individual is required one has no choice but to use wet weight measurements and accept that they provide a rough guide only. Studies on the growth of insects have emphasised that during an instar their linear dimensions and hence their volume remain constant, while their wet weight approximately doubles (CLARKE, 1956). Throughout this study, therefore, a twofold increase in wet

weight has been taken to represent true growth, allowing as it does for individual variation in gut content and body water content. Two other types of growth, 'reduced growth' and 'little or no growth at all', are recognised from wet weight measurements; these are defined in Section II of Chapter III.

The locusts were weighed on a torsion balance at 24 hr intervals and immediately before feeding so as to reduce short-term fluctuations in weight caused by individual variation in gut contents.

J. Vital staining with methylene blue

Newly moulted fifth instar nymphs were starved for several days and then injected with a reduced (leuco) solution of methylene blue according to the method of STARK et al. (1969) (after PANTIN, 1946).

0.15 ml of the reduced dye was injected into the head capsule through the dorsal neck membrane. After 1 hr the injected insect was decapitated, the head pinned out in a wax dish and a small window cut in the frons cuticle. The preparation was flooded with cold 8% ammonium molybdate and left for 24 hr at 0°C. The required portion of the anterior stomatogastric nervous system was then dissected out, washed

in distilled water, dehydrated, cleared and mounted. (A schedule of the procedure employed is presented in the Appendix.)

K. Electron microscopy

Animals were killed by decapitation and the head pinned out in a wax dish. A window was cut in the frons and the preparation flooded with ice-cold 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, containing 0.17 M sucrose. The tissues were fixed for 2 hr at 0°C and then washed overnight at 0°C in 0.1 M phosphate buffer, pH 7.4, containing 0.34 M sucrose. It was at this stage that the necessary tissues were dissected out and transferred to fresh buffer. The tissues were post-fixed at 0°C in 1% OsO_4 in 0.1 M phosphate buffer, pH 7.4, containing 0.34 M sucrose. Then followed another washing in buffer, dehydration in a graded series of ethanols, pre-staining in uranyl acetate and finally embedding in Araldite resin. (A schedule of the procedure employed is provided in the Appendix.)

Silver or gold sections were cut with a glass knife on a Servall Porter-Blum MT-2 ultramicrotome, expanded with trichloro-ethylene vapour and mounted on coated grids (200-mesh). Sections were post-stained with Reynold's lead citrate (REYNOLDS, 1963) and examined under an AEI EM6B electron microscope.

L. Radiography

The technique of contact radiography was first applied to ecdysis by CLARKE (1956) who used a standard clinical Watson 'Mobilix' X-ray machine and high contrast X-ray film. This same machine was used during the present study and gave good, reproducible results.

A high contrast X-ray film (Ilford Industrial G) was enclosed within a lead-backed, light-proof holder held at a distance of 75 cm from the lens. The locust to be X-rayed was laid on one side and held in position against the film holder by means of sellotape applied across its legs. Alternatively, the subject was X-rayed in a side-on position while clinging to muslin wrapped tightly round a narrow stick placed adjacent to the film holder. A standard exposure time of 0.2 sec at 43 kV and 60 mA was used throughout with the diaphragm fully open. The film was developed for 6 min in a high contrast X-ray developer (Ilford PQX-1) and fixed for 10 min in F 52 fixer.

M. Presentation and analysis of data

The raw data for the work presented in this thesis is recorded in tabular form in the Appendix. Each animal in a particular experiment is allotted a number and the numbers quoted in the text correspond to those in the Appendix. Abbreviations used in the text, in tables or in the Appendix are listed and defined at the beginning of the Appendix.

In view of the wide disparity between the initial weights of newly moulted locusts, particularly adults, body weight is

expressed as a percentage of that at the time of the operation. For graphical purposes the operation weight equals 100% and for tabular purposes it equals 0%.

Statistical analysis, where applied, took the form of STUDENT's 't' test, the values of the probability 'P' being obtained from "Statistical Tables for Biological, Agricultural and Medical Research" (FISHER and YATES, 1953). The 5% level of significance (P = 0.05) was adopted.

CHAPTER III

RESULTS

SECTION I

SURGICAL INTERFERENCE WITH THE ANTERIOR STOMATOGASTRIC NERVOUS SYSTEM IN FLEDGLING *Locusta*: ITS EFFECT UPON SOMATIC GROWTH

After the final ecdysis the locust undergoes a period of somatic growth during which protein, lipid and carbohydrate accumulate in the fat body and proteins collect in the haemolymph. Growth of the cuticle and flight muscles is especially noticeable at this time (HILL *et al.*, 1968). The somatic growth period continues until a maximum body weight, the 'basic weight' (NORRIS, 1954) is attained. In both *Locusta* (PHIPPS, 1950; STRONG, 1966, 1968) and *Schistocerca gregaria* (HILL *et al.*, 1968) the basic weight is reached approximately ten days after final ecdysis. In female locusts ovarian growth only occurs when materials become available after somatic growth has finished (HILL *et al.*, 1968).

Surgical interference with the anterior stomatogastric nervous system was first performed on newly moulted adults, the large size of the head capsule facilitating the various operations. Animals of both sexes were employed in this study. They were weighed daily up until Day 10 post-operative

by which time the control males had virtually attained a basic weight. All weight changes are recorded in Table I of the Appendix.

The experimental treatments were as follows: operated controls; one frontal connective cut; anterior, median and posterior pharyngeal nerves cut; recurrent nerve cut; both frontal connectives cut; recurrent nerve plus both frontal connectives cut; frontal ganglion removed. These treatments comprised three separate experiments, and since no differences were observed between the control groups for the three experiments the results are combined. Operative technique and post-operative care followed the pattern set out in Chapter II. For identification purposes each animal was marked with cellulose dope in the region of the pronotum. Unless otherwise stated, the graphs indicate the mean daily weight changes of those animals surviving to Day 10, with the operation weight equalling 100%.

One frontal connective cut (6 m, 6 f)

It can be seen from Fig. 1 that the cutting of one frontal connective had no adverse effect upon somatic growth when compared to the controls.

Anterior, median and posterior pharyngeal nerves cut (5 m, 7 f)

The anterior and posterior pharyngeal nerves have been described in Locusta by CLARKE and LANGLEY (1963b). According to ROOME (1968) a pair of median pharyngeal nerves leave the frontal ganglion, between the anterior and posterior pairs, and branch to the dorsal dilator muscles of the pharynx and

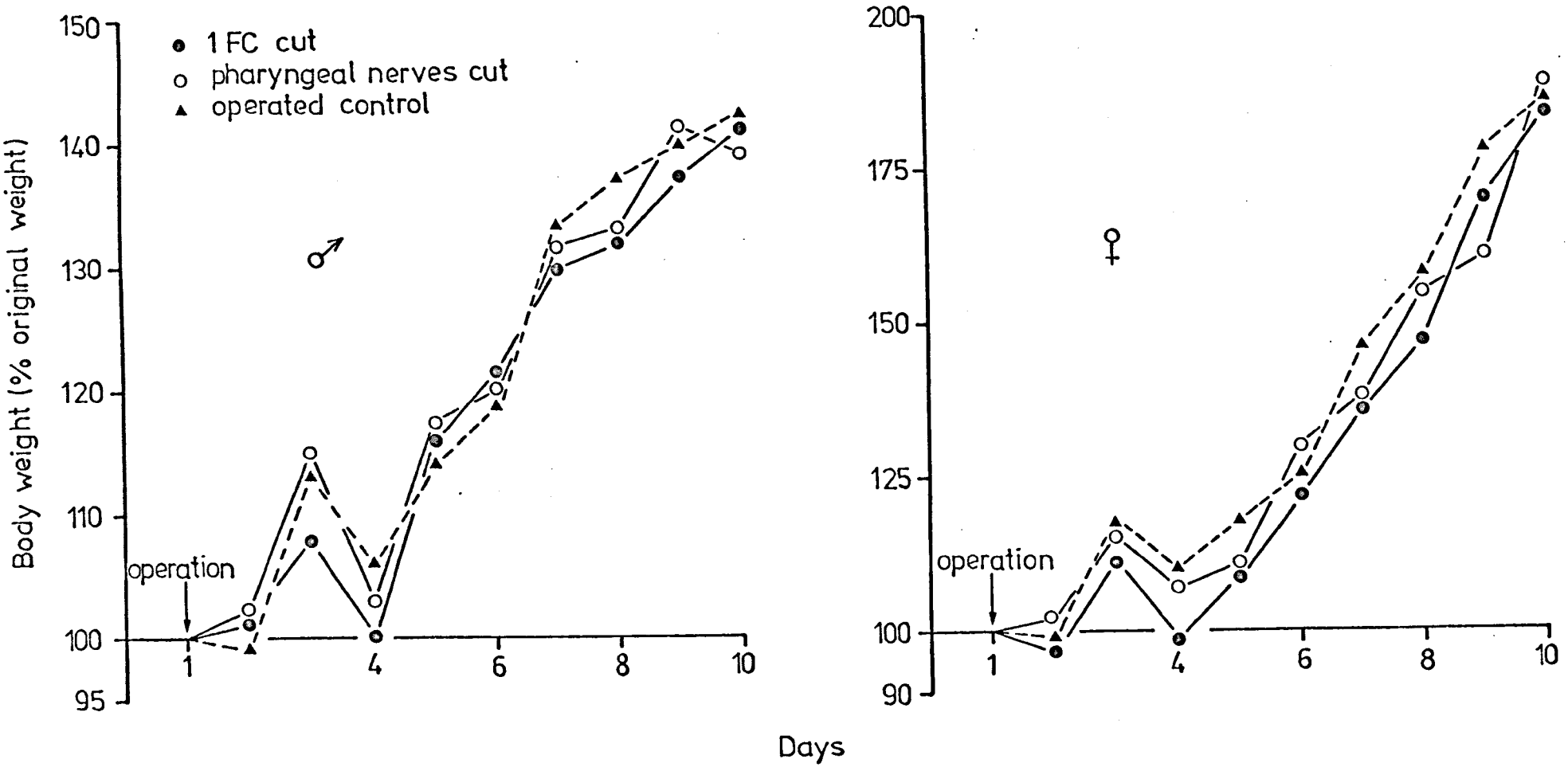


FIG 1. Growth after severance of one frontal connective, and after severance of anterior, median and posterior pharyngeal nerves.

to the muscular coat of the pharynx. The morphology of these nerves is considered in Section IV.

Somatic growth was unaffected by severance of the anterior, median and posterior pharyngeal nerves, the growth curves of the operated animals being no different from those of the controls (Fig. 1).

Recurrent nerve cut (6 m, 8 f)

The cutting of the recurrent nerve led to a reduced growth rate among the operated animals when compared to the controls (Fig. 2).

In the blowfly, Phormia regina (DETHIER and BODENSTEIN, 1958; DETHIER and GELPERIN, 1967) and in adult male Schistocerca (FRASER ROWELL, 1963) the operation induces hyperphagia, which may also be its effect in adult Gryllus bimaculatus (ROUSSEL, 1966). Recurrent nerve severance inhibits crop emptying in adult Melanoplus differentialis (DOGRA and EWEN, 1971) and also in Leucophaea maderae (SCHARRER, 1945; ENGELMANN, 1968; TAYLOR, 1969).

Both frontal connectives cut (14 m, 15 f)

The 21 operated animals surviving to Day 10 can be divided into two groups: those maintaining a constant weight (14), and those showing fairly considerable weight increases (7) which, in the majority of cases, are lower than those of the controls (Fig. 3).

In young adult Gryllus the cutting of both frontal connectives brings about a rapid early death (ROUSSEL, 1966), while in adult female Melanoplus the operation inhibits crop emptying (DOGRA and EWEN, 1971).

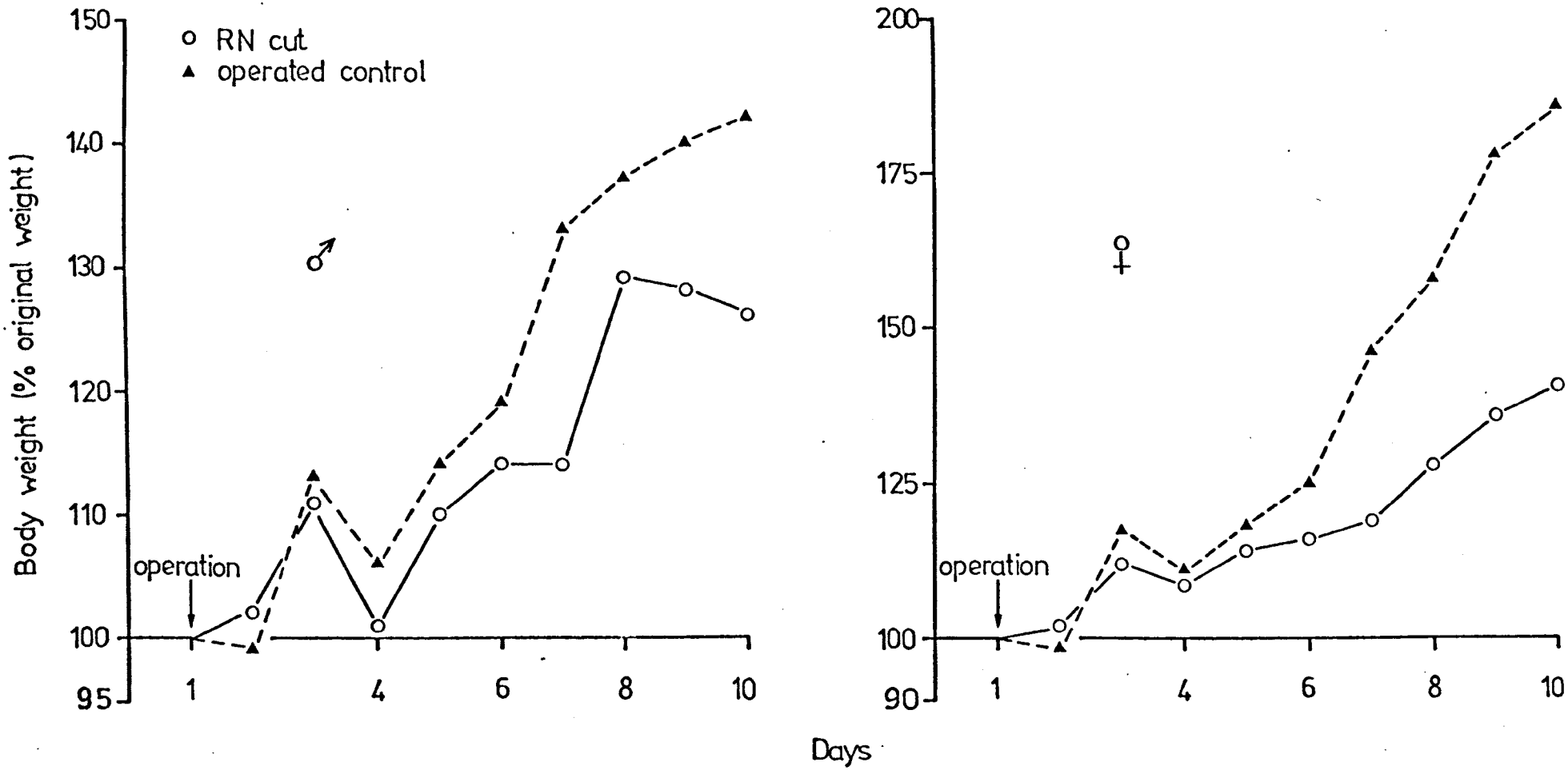


Fig 2. Growth after severance of the recurrent nerve.

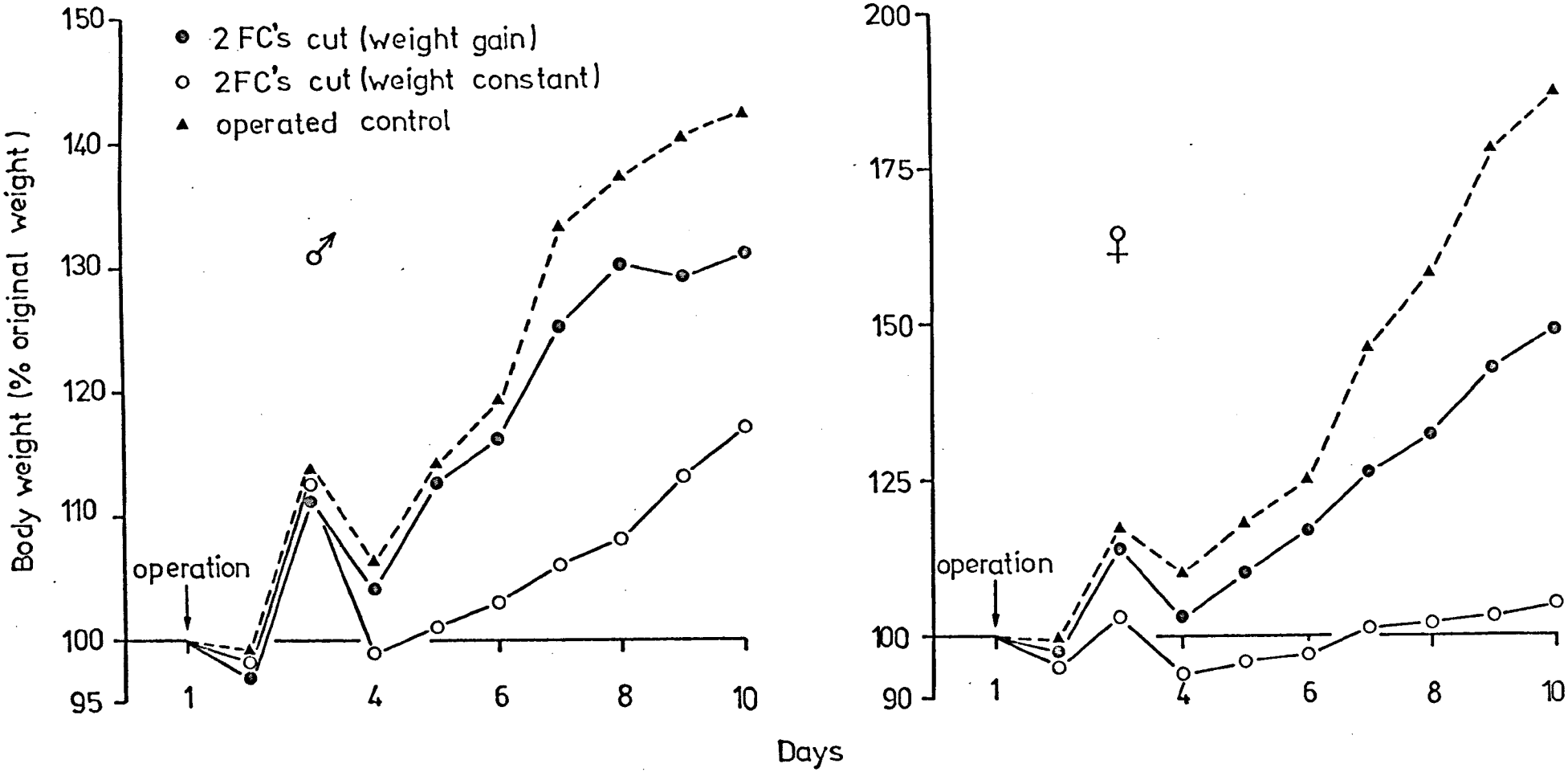


FIG 3. Growth after severance of both frontal connectives.

Recurrent nerve plus both frontal connectives cut (14 m, 15 f)

The operation was attended by an early high mortality with only 8 animals surviving to Day 10. 3 of these maintained a constant weight throughout the experimental period and the remaining 5 exhibited fairly considerable weight increases which were, however, lower than those of the controls (Fig. 4).

Removal of the frontal ganglion (22 m, 24 f)

Mortality was also high after frontal ganglionectomy, 34 operated animals dying before Day 10. The vast majority of these animals died at weights which were lower than those recorded at the time of the operation. The 12 animals surviving to Day 10 can be divided into three groups (Fig. 5): those losing weight (5 animals); those maintaining a constant weight (6 animals); and those showing fairly considerable weight increases (1 animal).

Adult Locusta (CLARKE and LANGLEY, 1963; STRONG, 1966), adult Schistocerca (HILL et al., 1966) and adult Melanoplus (GILLOTT et al., 1970) maintain a constant weight after frontal ganglionectomy. Immature adult Gryllus (ROUSSEL, 1966) either maintain a constant weight or lose weight after the operation, but survive for a limited period only. Frontal ganglionectomy in adults of the beetle Necrophorus vespillo, on the other hand, has no effect upon growth (ROUSSEL, 1966). The majority of workers (HIGHNAM et al., 1966; ROUSSEL, 1966; GILLOTT et al., 1970; DOGRA and EWEN, 1971) conclude that the operation interferes with crop emptying, causing food to accumulate in the foregut.

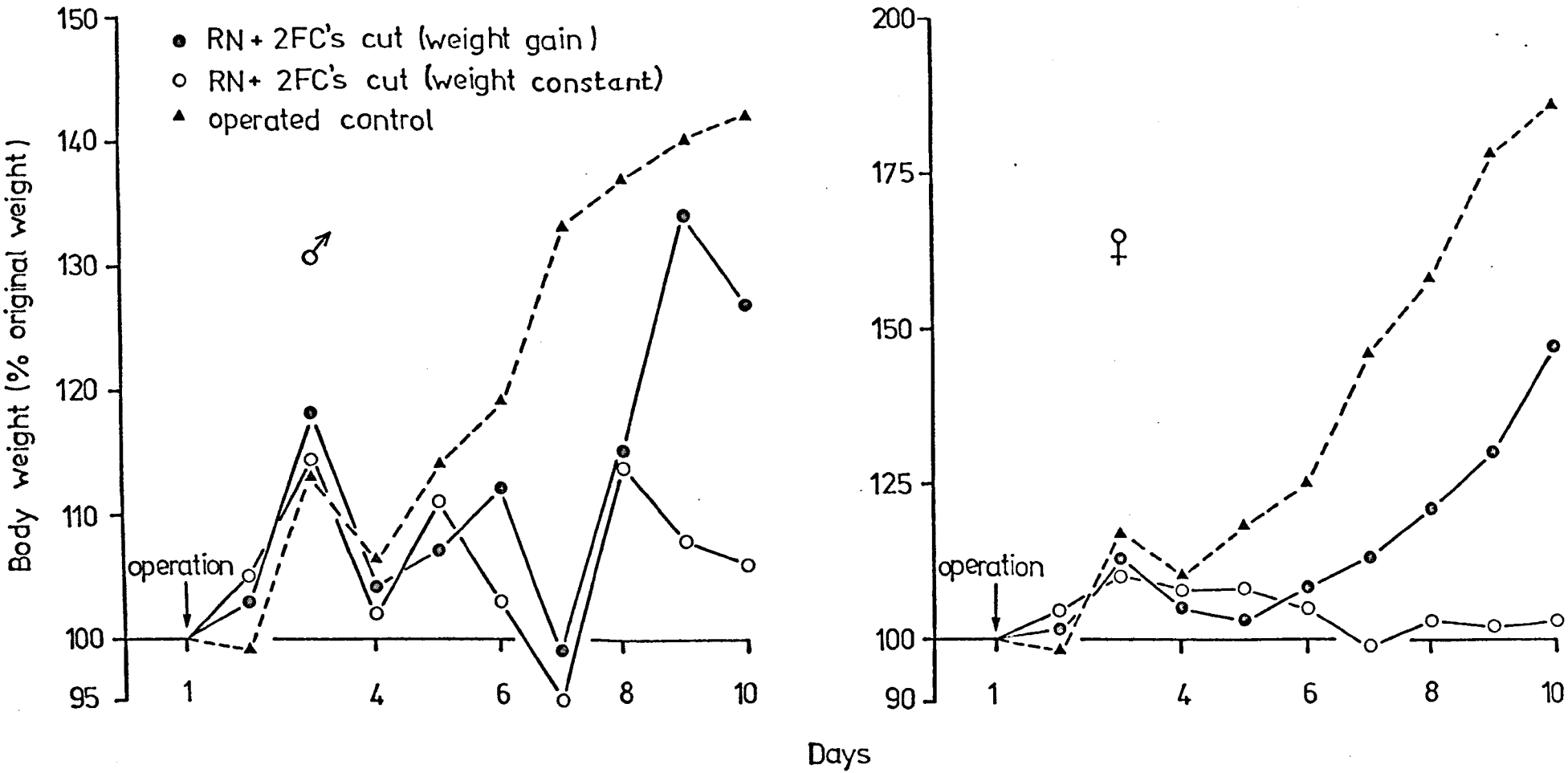


FIG 4. Growth after severance of recurrent nerve plus both frontal connectives.

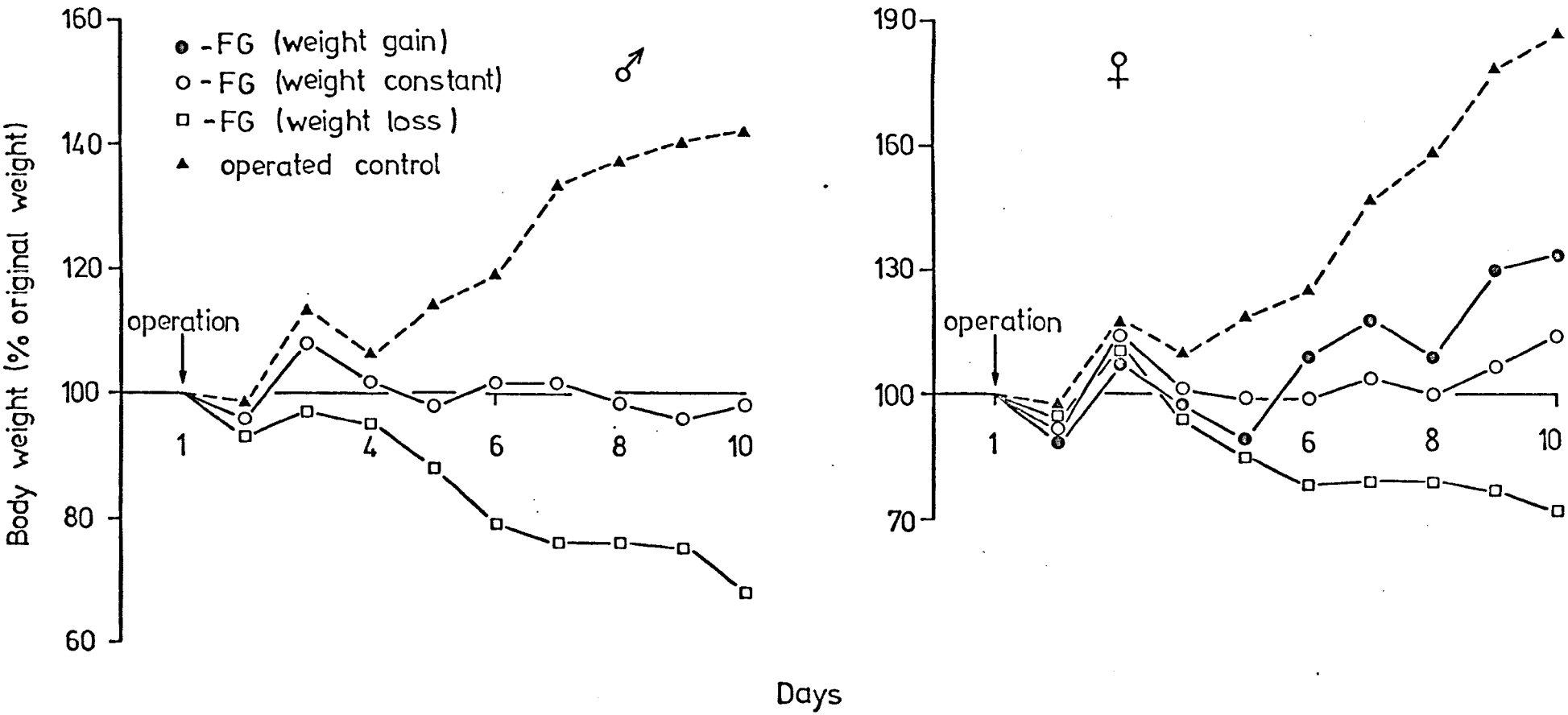


FIG 5. Growth after removal of the frontal ganglion.

It is clear that the separation of the frontal ganglion from the surface of the foregut is not a growth arresting operation when performed in adult animals. Neither does the severance of both frontal connectives, or the severance of the recurrent nerve plus both frontal connectives block growth in every operated animal. The results of the latter operation suggest that alternative nervous pathways to those outlined by CLARKE and LANGLEY (1963c, d) are relaying sensory information from the foregut to the brain and corpora cardiaca. One possibility is the hypocerebral ganglion ⟶ NCC I route of STRONG (1966).

The effect on growth of surgical interference into the anterior stomatogastric nervous system is considered further in Section II, where the majority of the above operations are performed on newly moulted fifth instar nymphs.

SECTION II

SURGICAL INTERFERENCE WITH THE ANTERIOR STOMATOGASTRIC NERVOUS SYSTEM IN FIFTH INSTAR *Locusta*: ITS EFFECT UPON GROWTH AND MOULTING

JOUSSET DE BELLESME (1877) was the first to implicate air in the process of ecdysis. He showed that the pronounced enlargement of freshly emerged dragon-flies was accomplished by internal air pressure built up, not in the main tracheae, but in the digestive tract. Similar observations have since been made in many insect species, including the two acridids, *Stauronotus maroccanus* (KUNKEL D'HERCULAIS, 1890) and *Locusta migratoria* (DUARTE, 1939).

Although TESTENOIRE and LEVRAT were using X-rays in the field of insect investigation as long ago as 1896, sixty years were to elapse before the technique was first applied to ecdysis. CLARKE (1956) found that the locust, with its thick body, was an ideal subject for the differential absorption of X-rays, and from radiographs obtained he was able to determine the function of the dorsal abdominal air sacs during ecdysis and subsequent instar development. The same radiographic technique is used in the present investigation to determine the effect of various surgical interruptions into the anterior stomatogastric nervous system on the processes of gut emptying and air swallowing at ecdysis. The radiographs of operated animals presented in the text depict the maximum amounts of air that could be observed in the gut during attempted ecdysis.

The effect of these surgical interruptions on growth is measured as daily change in body weight, and the weight measurements of all operated animals are recorded in Tables II to IX of the Appendix. Three types of growth are recognized:

(i) True growth - a twofold or more increase in the operation weight. Control animals fit into this category, and the mean maximum percentage weight increases of operated and control animals exhibiting true growth are compared for each operation.

(ii) Reduced growth - 50 to 100% increase in the operation weight.

(iii) Little or no growth - 0 to 50% increase in the operation weight.

Newly moulted animals of both sexes were employed in this study. The techniques of microsurgery and radiography, together with the methods of sampling experimental insects, have been described in Chapter II. Unless otherwise stated the growth curves in Figs. 6 to 13 are for those animals moulting (or attempting to moult) on the day by which 50% of the operated population have undergone ecdysis (or attempted ecdysis). In Tables 2 to 9 the operation weight equals 0%, while in Figs. 6 to 13 it equals 100%.

Experiment 1

X-ray analysis of operated control fifth instar locusts over a period covering the final moult

Four operated control fifth instar nymphs were X-rayed at regular intervals from the time feeding ceased prior to ecdysis until two days after the final moult when feeding was well under way again. A selected sequence of radiographs taken from one particular individual is presented as Plates 1-24. In a recent paper VINCENT (1971) conveys a time scale, based on casual observation, of superficial changes taking place during the final ecdysis in Locusta. This was found to correspond reasonably well with the scale of external changes observed in animals of the present experiment.

(Note: The X-ray machine was housed in a room whose ambient temperature was some 5°C below that of the insectary. The time sequence presented below may, therefore, represent a slight exaggeration of what happens under normal insectary conditions.)

Plates 1 and 2

The first bubble of air appears in the foregut 32 hr before ecdysis. Approximately 24 hr later the amount of air in the crop has increased but air is still absent from the hindgut. Digestion of the old endocuticle at this time makes the locust soft to touch.

PLATES 1-24. X-ray photographs of an operated control fifth instar locust during the final ecdysis

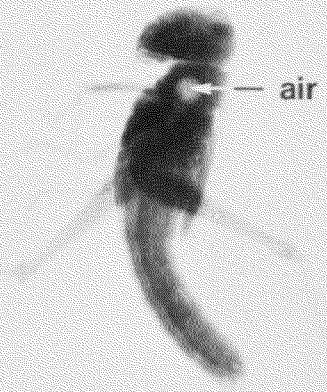
PLATE 1. 32 hours before final escape from the old cuticle.

PLATE 2. 9 hours before final escape from the old cuticle.

PLATE 3. 4 hours before final escape from the old cuticle.

PLATE 4. 3 hours before final escape from the old cuticle.

PLATES 1-4



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Plates 3, 4 and 5

Air is first observed in the hindgut 4 hr before ecdysis. The amount increases during the next hour and 1 hr before moulting even more air is present in the hindgut.

Plate 6

30 min before ecdysis the locust alters its position on the perch to one where the head points downwards so that maximum use of gravity can be made during moulting. Longitudinal contractions of the abdomen at this time are very obvious as air continues to enter the hindgut.

Plates 7 and 8

The wing pads begin to separate 20 min before ecdysis and 4 min later they are fully apart. Separation of the wing pads is taken to represent the start of ecdysis (VINCENT, 1971). The foregut expands as more air is swallowed and air is visible in the midgut caeca. Flexing movements of the abdomen at this time are very intense.

Plates 9, 10, 11 and 12

The haemolymph pressure, which is already at an elevated level on account of the high blood volume of locusts at this time (LEE, 1961), is raised still further by the continued expansion of the foregut. The high blood pressure and the actively contracting body muscles together cause the old cuticle to split along predetermined ecdysial lines of weak-

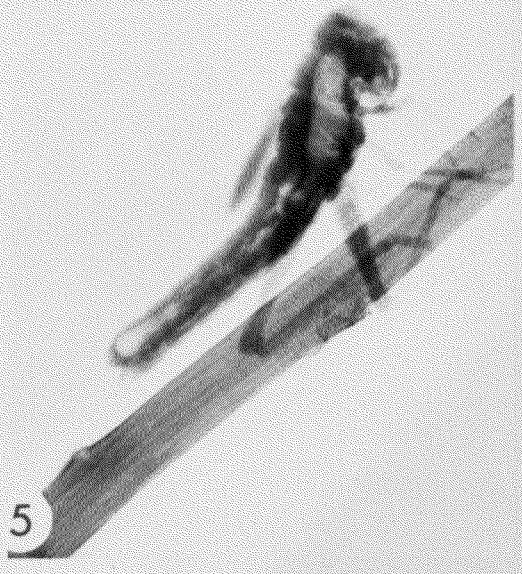
PLATE 5. 1 hour before final escape from the old cuticle.

PLATE 6. 30 minutes before final escape from the old cuticle.

PLATE 7. 20 minutes before final escape from the old cuticle.

PLATE 8. 16 minutes before final escape from the old cuticle.

PLATES 5 – 8



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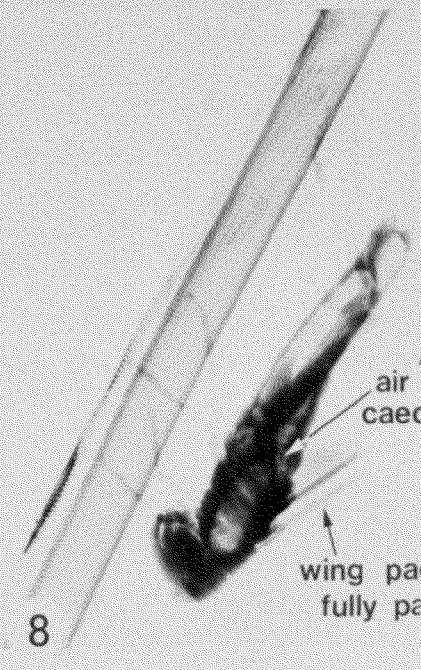

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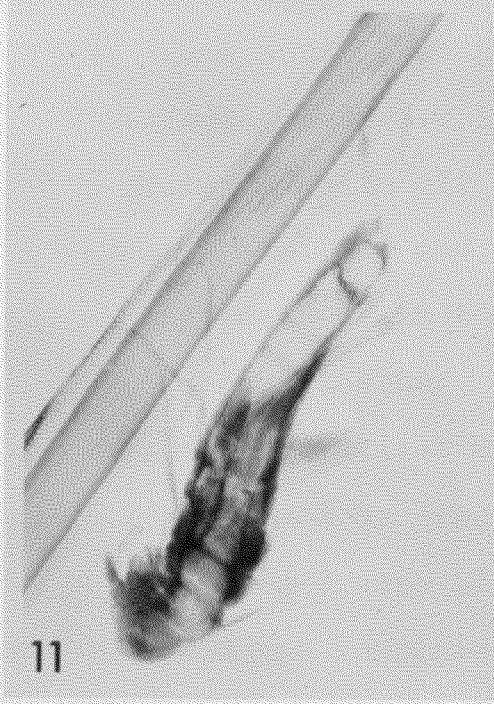
PLATE 9. 14 minutes before final escape from the old cuticle.

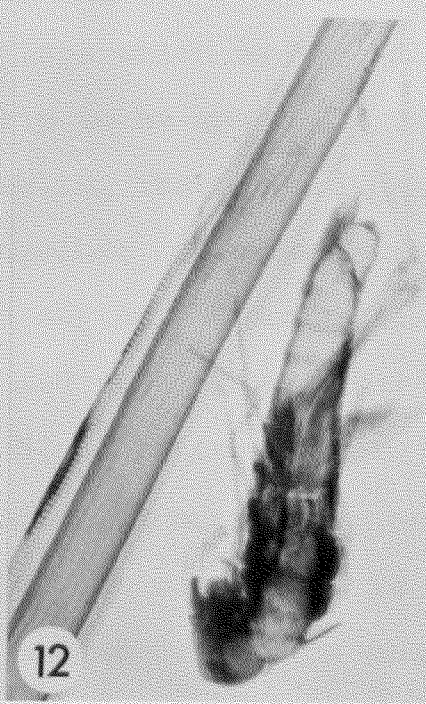
PLATE 10. 12 minutes before final escape from the old cuticle.

PLATE 11. 9 minutes before final escape from the old cuticle.

PLATE 12. 6 minutes before final escape from the old cuticle.

PLATES 9 – 12





ness situated in the mid-dorsal region of the thorax and along the epicranial suture of the head (DUARTE, 1939).

Plates 13 and 14

As the head and thorax emerge so the shed cuticle of the mouthparts becomes visible. The wings are withdrawn from the old wing pad cuticle and the gut reaches its maximum state of distension at this time. The limbs finally escape and the locust remains suspended from the old cuticle by the tip of the abdomen, for a period of 7 min, before falling to the ground. This rest period is most probably associated with preliminary hardening of the legs (VINCENT, 1971).

Plates 15, 16, 17 and 18

The newly emerged adult quickly regains the perch using only the front and middle pairs of legs which by this time are sufficiently hard for a firm grip to be applied. The gut remains distended with air thus enabling the blood to act as a hydrostatic skeleton and this, together with continuing muscular contractions in the region of the abdomen, facilitates expansion of the new cuticle and unfolding of the hypodermis to its fullest extent. Blood is forced into the wings which respond by gradually unfolding until they are completely expanded some 40 min after the moult. It is clear that some food has remained in the midgut during the moult. The midgut is the only region of the gut where food could be retained since the linings of both the foregut and hindgut are moulted.

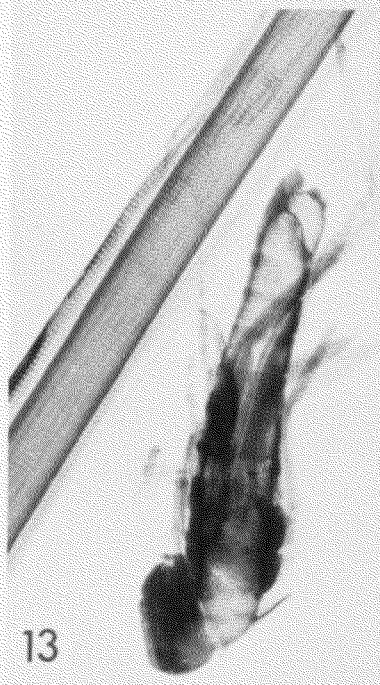
PLATE 13. 4 minutes before final escape from the old cuticle.

PLATE 14. 1 minute before final escape from the old cuticle.

PLATE 15. 10 minutes after final escape from the old cuticle.

PLATE 16. 20 minutes after final escape from the old cuticle.

PLATES 13 – 16



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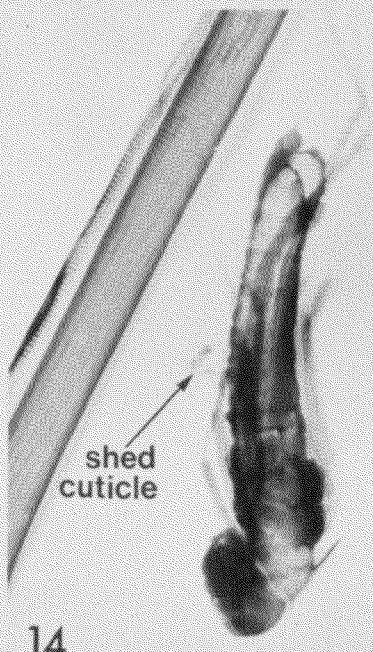

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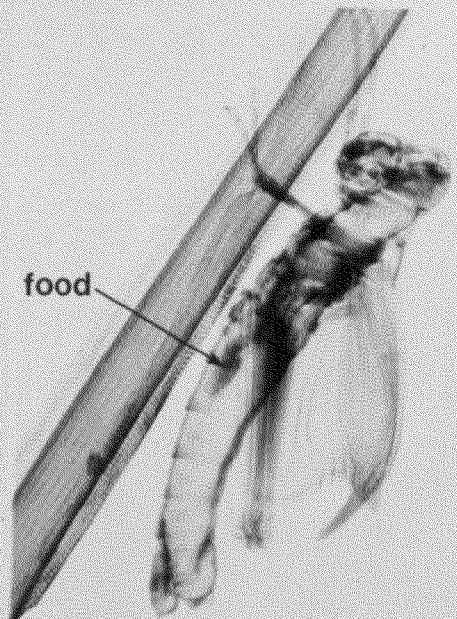

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PLATE 17. 25 minutes after final escape from the old cuticle.

PLATE 18. 43 minutes after final escape from the old cuticle.

PLATE 19. 1¼ hours after final escape from the old cuticle.

PLATE 20. 2 hours after final escape from the old cuticle.

PLATES 17 – 20

17

18

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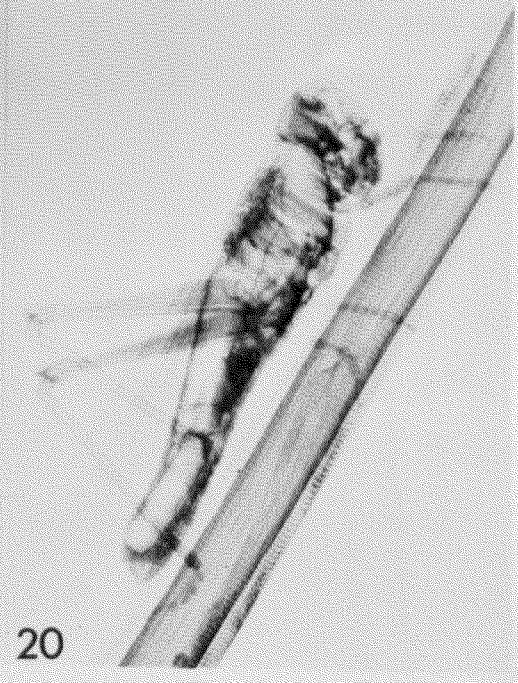

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Plate 19

The wings are folded up to their normal resting position once expansion is complete (1¼ hr after ecdysis) and as they harden so they become more difficult to visualise in the ensuing radiographs.

Plates 20, 21 and 22

The foregut has begun to collapse 2 hr after ecdysis and by the time 6 hr have elapsed it has assumed more normal dimensions. 12 hr after the moult the hindgut has expelled all but the last bubble of air. The fully expanded air sacs are now clearly visible. CLARKE (1956) states that the abdominal air sacs of the locust form a system permitting changes of volume at an ecdysis when the mass and density of the tissues remain constant. The air sacs are gradually obliterated by the developing tissues so that by the end of the stadium they have become completely occluded.

Plate 23

The first meal is taken 12-24 hr after ecdysis and food can be detected along the length of the gut at 24 hr. The S-bend of the colon, mentioned by ALBRECHT (1953) and figured in drawings from radiographs by GOODHUE (1963), is clearly visible.

PLATE 21. 6 hours after final escape from the old cuticle.

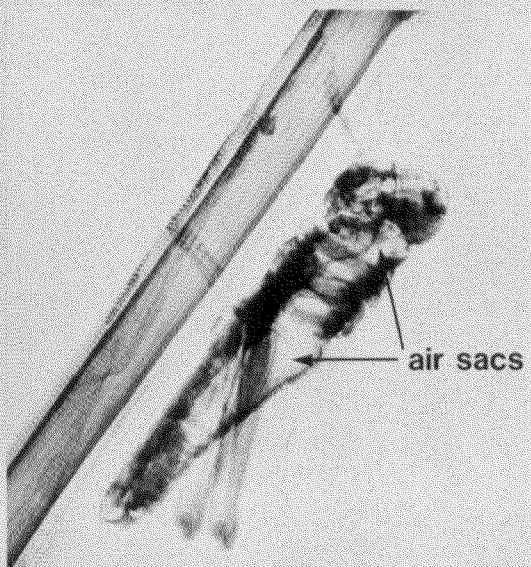
PLATE 22. 12 hours after final escape from the old cuticle.

PLATE 23. 24 hours after final escape from the old cuticle.

PLATE 24. 48 hours after final escape from the old cuticle.

PLATES 21–24

21



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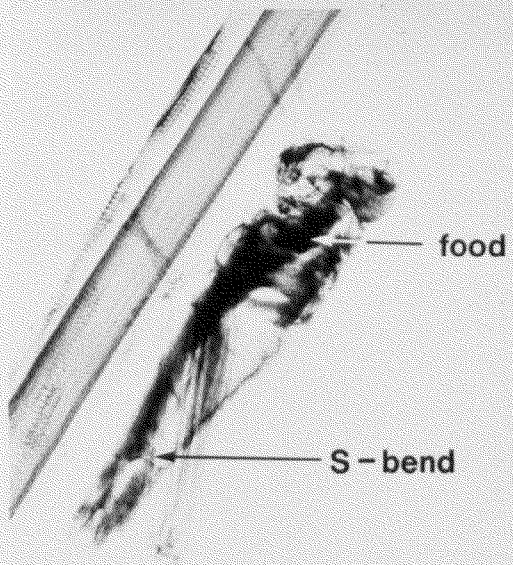

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Plate 24

Even more food is present in the crop and midgut and the S-bend in the colon is again apparent. The adductor muscles of the mandibles are also very obvious.

Summary

Air is first detected in the foregut 32 hr before the moult. 28 hr later it appears in the hindgut. This sort of timing is to be expected since the foregut will be emptying and swallowing well before the hindgut has removed the last traces of food and begun to take in air through the anus. The gut reaches its maximum state of distension several minutes before final escape from the old cuticle is accomplished. Dilation is maintained for 1-2 hr after moulting, thus allowing unfolding of the hypodermis and expansion of the new cuticle to take place. By 12 hr after ecdysis the gut has assumed more normal dimensions and the first meal is taken 12-24 hr after the moult.

Experiment 2

The effect of cutting the recurrent nerve on growth and moulting

The experimental treatments were as follows:

(a) Recurrent nerve cut 14 animals

(b) Operated controls 8 animals

Results

Sectioning the recurrent nerve produced two quite distinct responses:

(i) 50% of the operated animals died before Day 10 and in every individual the foregut, midgut and most of the hindgut were packed full of undigested grass at autopsy (Table 1, Plate 25).

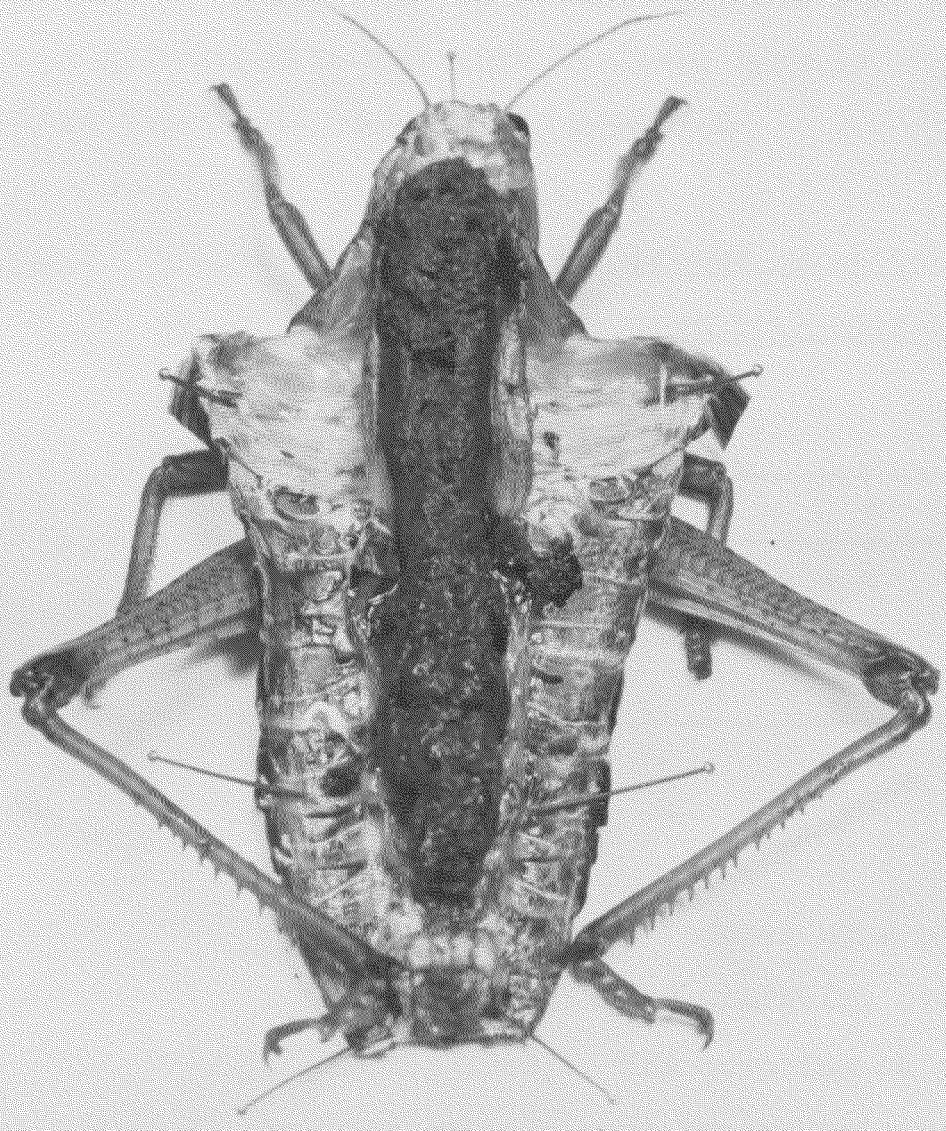
TABLE 1. Gut contents of response (i) operated animals at autopsy

Animal No.	Food content of gut				
	Crop	Midgut	Hindgut		
			Ileum	Colon	Rectum
1	+++++	+++++	+++++	++++	+
3	+++++	+++++	+++++	+++	+
4	+++++	+++++	+++++	+++	-
8	+++++	+++++	+++++	++	-
9	+++++	+++++	++++	+	-
11	+++++	+++++	+++++	+	-
14	+++++	+++++	+++++	++++	+

\+ very little food +++++ gut distended with food

PLATE 25. Dorsal dissection of a recurrent nerve cut fifth instar locust dying before Day 10. Note the presence of vast quantities of food in the foregut, midgut and much of the hindgut.

PLATE 25



These animals did not produce any faeces and the midgut caeca were frequently shrunken in appearance and squashed against the body wall by the excessive amount of grass in the midgut.

(ii) The other 50% operated animals grew at a reduced rate compared to the controls and moulted to adults, on average one day later (Fig. 6). Table 2 shows that the mean maximum percentage weight gain of these animals was significantly lower than that of the controls.

TABLE 2. Comparison of the mean maximum percentage weight increases of response (ii) operated animals and the controls

Treatment	Individuals	Mean	S.E.	t	'P'
RN cut, response (ii)	7	145	4	6.00	0.001
Controls	8	175	3		

Conclusions

Recurrent nerve severance frequently induces hyperphagia (DETHIER and BODENSTEIN, 1958; DETHIER and GELPERIN, 1967; GELPERIN, 1967; FRASER ROWELL, 1963). The question as to whether response (i) operated animals were hyperphagic must remain open since they were not made the subject of any quantitative food measurements. Their gut condition is identical to that of adult Gryllus bimaculatus (ROUSSEL, 1966) after recurrent nerve severance. In both cases death

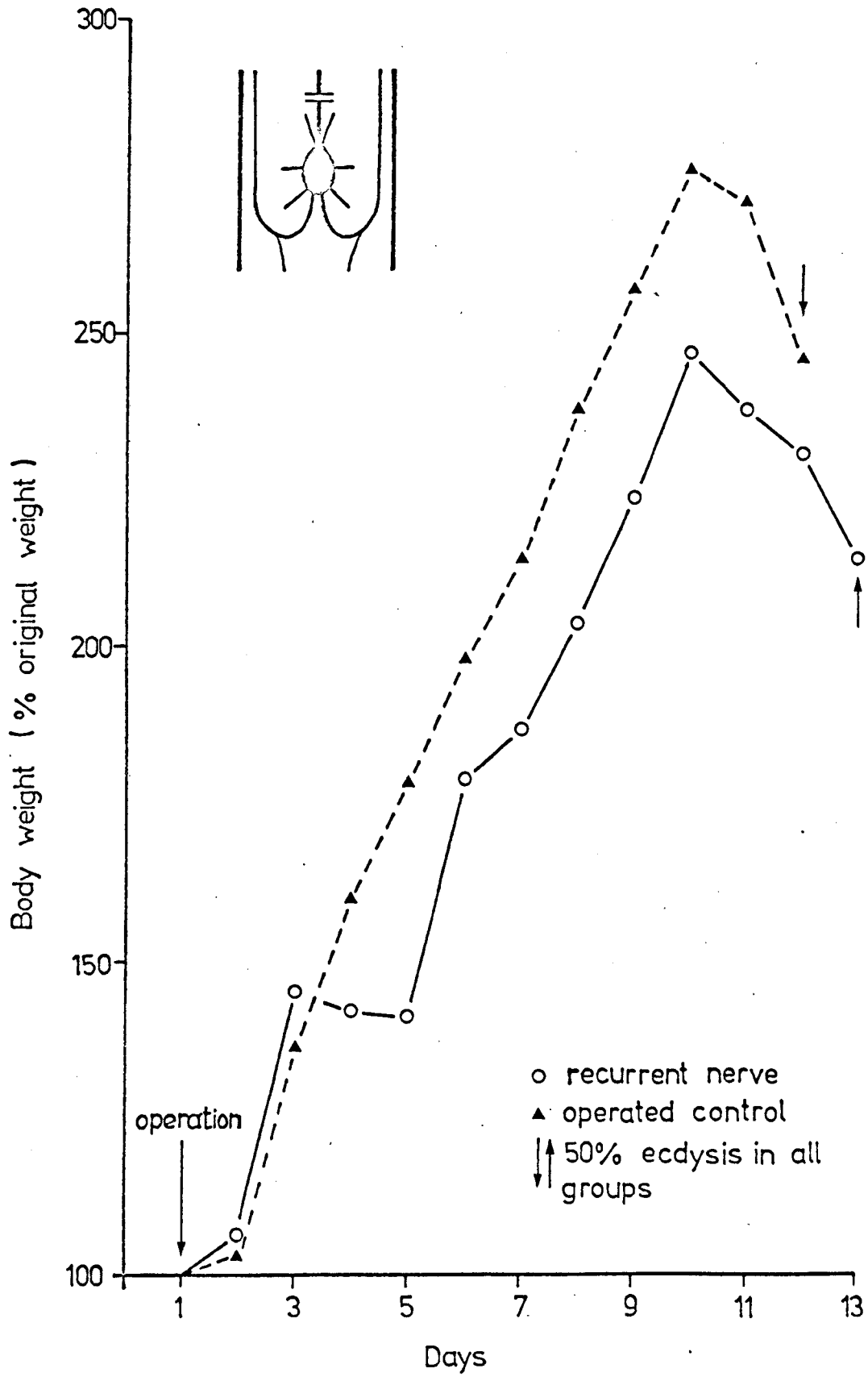


FIG 6. Growth after severance of recurrent nerve.

is ultimately due to lack of metabolites entering the haemolymph, the operation having an adverse effect upon the digestive mechanisms of these insects. The remaining (response (ii)) operated animals behave in a similar manner to the third instar nymphs of CLARKE and LANGLEY (1963b), growing at a reduced rate compared to the controls and showing a slight delay in moulting.

Experiment 3

The effect of cutting one frontal connective (proximal or distal to branches) on growth and moulting

ROOME (1968) makes brief mention of fine nerves leaving each frontal connective in fourth instar Locusta. Intra-vitam injection of methylene blue and subsequent dissection confirmed the existence of these nerves in fifth instars. The fine nerves, usually three in number, leave each frontal connective in the region where this nerve passes between the retractors of the mouth angle and the posterior retractors of the labrum. Sensory cell bodies, lying on the dorsal surface of the muscular coat of the pharynx, were seen to be associated with some of the fine nerve branches; motor nerve endings to some of the nerve branches were also apparent. The neuromorphology of this region of the stomatogastric nervous system is considered in more detail in Section IV. Suffice it to say here that sensory information from the pharynx can be relayed to the brain and/or corpora cardiaca via the fine nerve branches of the frontal connectives as

well as via the anterior and posterior pharyngeal nerves (LANGLEY, 1962; CLARKE and LANGLEY, 1963b, c).

The treatments in Experiment 3 were as follows:

(a) One frontal connective cut (proximal to branches) — 16 animals

(b) One frontal connective cut (distal to branches) — 14 animals

(c) Operated controls — 18 animals

Results

Animals of treatments (a) and (b) grew at a similar rate to the controls, with treatment (b) animals tending to moult on average one day later than those in the other two groups (Fig. 7). Table 3 shows that the mean maximum percentage weight increases of the operated and control groups were not significantly different. None of the operated animals leaked digestive fluid at any time during the experiment.

TABLE 3. Comparison of the mean maximum percentage weight increases of treatments (a), (b) and (c) animals.

Treatment	Individuals	Mean	S.E.		t	'P'
(a) 1 FC cut (proximal)	14	176	4	a:b	0.71	0.5-0.4
(b) 1 FC cut (distal)	12	172	4	a:c	1.00	0.4-0.3
(c) Control	16	171	3	b:c	0.20	0.9-0.8

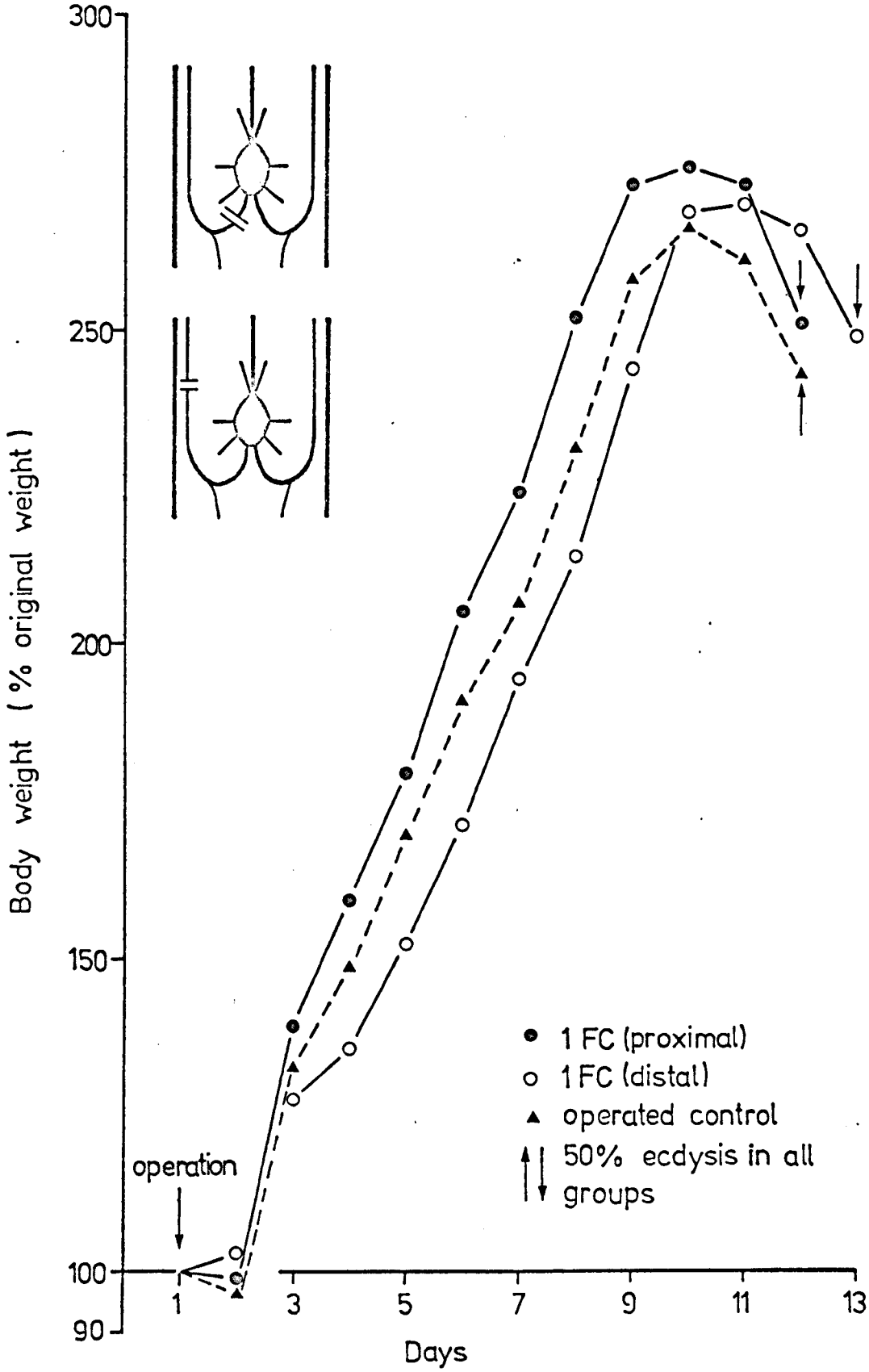


FIG 7. Growth after severance of one frontal connective (proximal), one frontal connective (distal).

Conclusions

In accordance with CLARKE and LANGLEY (1963c), the cutting of one frontal connective has no adverse effect upon the growth and moulting cycle.

Experiment 4.

The effect of cutting two frontal connectives (proximal to branches) on growth and moulting

The experimental treatments were as follows:

(a)	Both frontal connectives cut (proximal to branches)	47 animals
(b)	Operated controls	20 animals

Results

The 39 operated animals surviving beyond Day 6 can be divided into five groups according to their growth and moulting responses:

(i) Growth and attempted moulting

15 animals exhibited true growth and died between Days 15 and 20 while attempting to moult to adults. The growth rate of these animals was much lower than that of the controls and the attempted moult was made on average five days after the controls had successfully moulted (Fig. 8). Table 4 shows that the mean maximum percentage weight increase of group (i) operated animals was significantly lower than that of the controls.

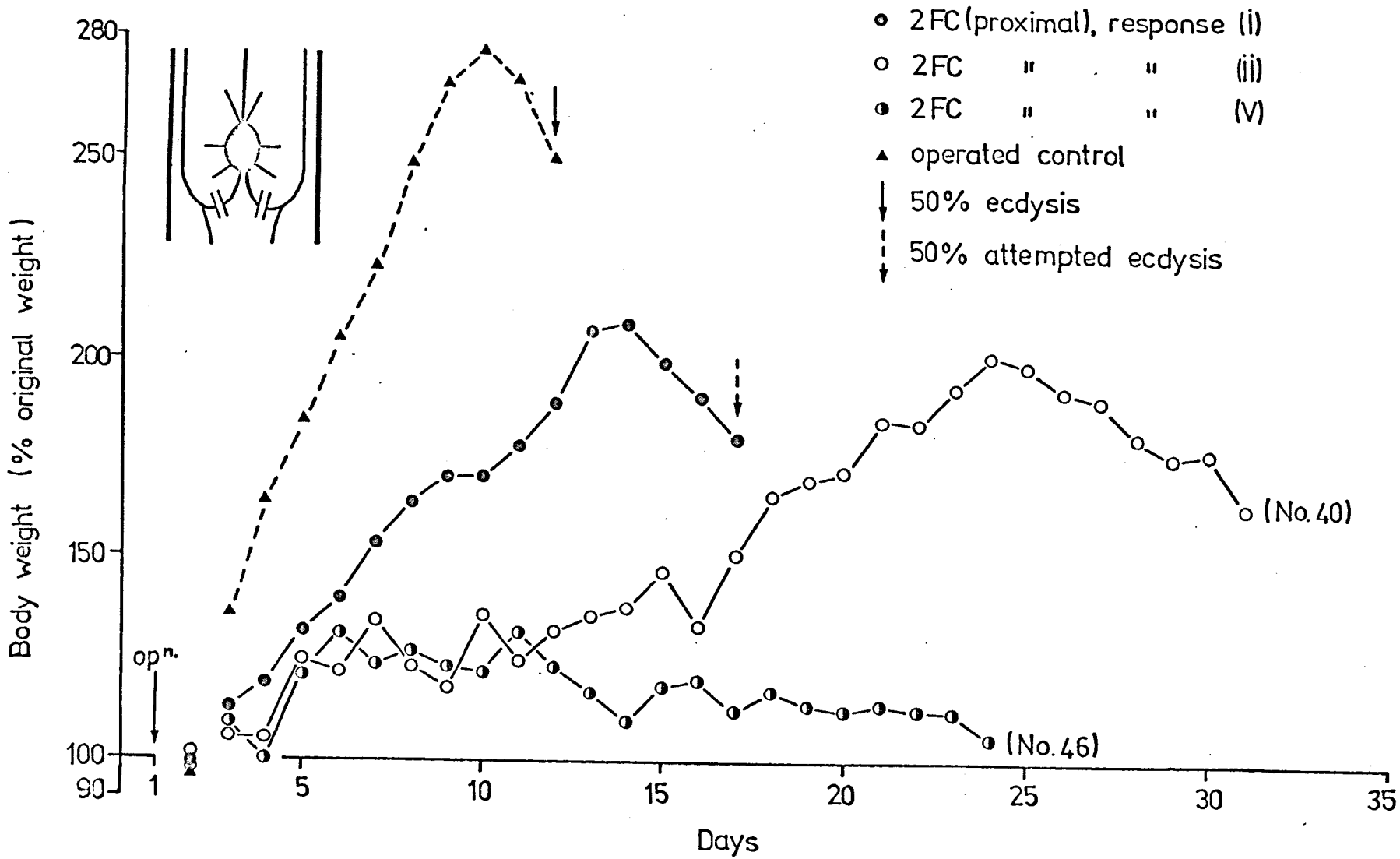


FIG 8. Growth after severance of two frontal connectives (proximal).

TABLE 4. Comparison of the mean maximum percentage weight increases of group (i) operated animals and the controls

Treatment	Individuals	Mean	S.E.	t	'P'
2 FC's cut (proximal) group (i)	15	117	4	13.87	0.001
Controls	16	179			

The operated locust adopted a normal posture for moulting, with the head pointing downwards, but after several hours of endeavouring to escape from the old cuticle the exhausted animal fell to the floor and eventually died. The wing pads of some operated animals at the time of death were widely parted and the abdomen drawn up by approximately 5 mm from the tip of the old cuticle (Plate 26), while in others abdominal retraction only was manifest (Plate 27).

8 operated locusts were X-rayed during their attempted moults and a representative sequence of radiographs from one particular individual (No. 37) is presented as Plates 30-32. It is obvious that reduced amounts of air are present in the gut compared to the controls (Experiment 1). More air can be detected in the gut of animal No. 21 (Plate 33), but there was evidently still not enough for a successful moult. Air continued to enter the hindgut after the operation. Post-mortem examination of group (i) animals showed that food was usually absent from the crop, colon and rectum, but was present in the midgut and ileum.

PLATE 26. Fifth instar nymph subjected to severance of both frontal connectives (proximal). Note the retracted abdomen and widely parted wing pads.

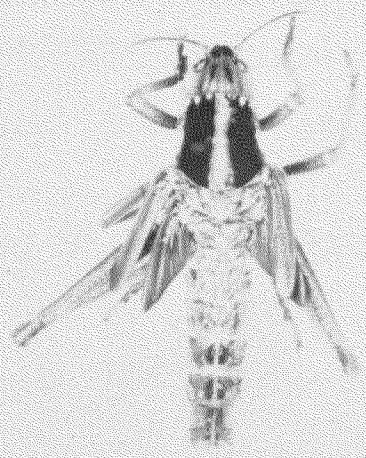
PLATE 27. Fifth instar nymph subjected to severance of both frontal connectives (proximal). Note the retracted abdomen.

PLATE 28. Operated control fifth instar nymph 1 hour before final escape from the old cuticle.

PLATE 29. Fifth instar nymph deprived of the frontal ganglion. Note the shrunken abdomen.

(N.B. Operated animals in Experiments 5 and 6 at the time of attempted moulting were similar in external appearance to those animals in Plates 26 and 27.)

PLATES 26 – 29



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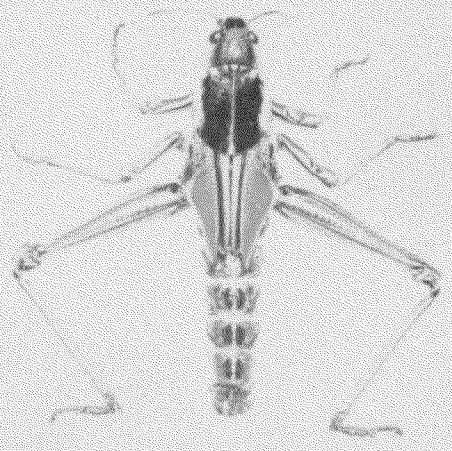

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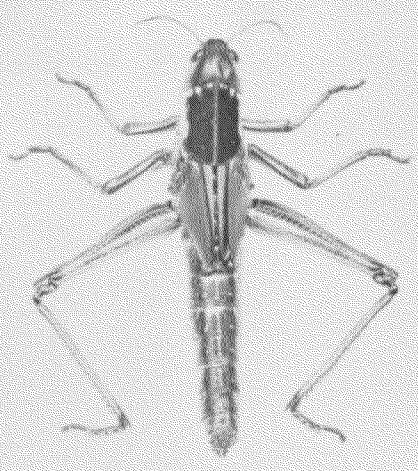

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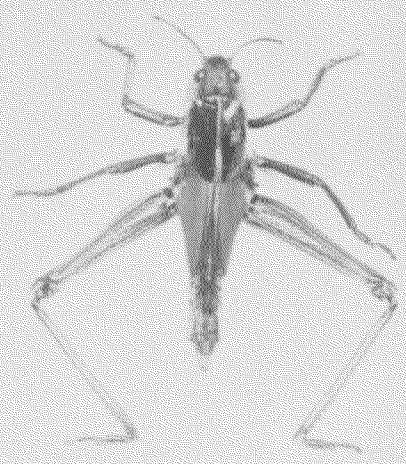

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PLATES 30-32. X-ray photographs, taken over a period covering the attempted moult, of a fifth instar locust subjected to severance of both frontal connectives (proximal).

PLATE 30. 4½ hours before the attempted moult.

PLATE 31. At the time of attempted moulting.

PLATE 32. 5 hours after the attempted moult.

PLATE 33. X-ray photograph, taken at the time of attempted moulting, of both frontal connectives cut (proximal) fifth instar nymph (No. 21).

PLATES 30 – 33

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(ii) Growth and no attempted moulting

Animal No. 40 increased its operation weight by a maximum of 101% and survived for 31 days (approximately three times the normal instar length) without ever forming a new cuticle. It can be seen from Fig. 8 that very little growth occurred during the first sixteen days post-operative, but that thereafter the growth curve resembled that of group (i) animals attempting to moult on Day 17.

(iii) Reduced growth and attempted moulting

Operated animals Nos. 38 and 45 showed maximum weight increases of 82% and 76% respectively before dying on Day 17 while attempting to moult to adults.

(iv) Reduced growth and no attempted moulting

7 animals exhibited maximum weight increases that were 50-100% above their operation weights. None of them ever developed a new cuticle.

(v) Little or no growth and no attempted moulting

The remaining 14 animals maintained a fairly constant weight until death and failed to develop a new cuticle. The weight changes of one such individual (No. 46) are illustrated in Fig. 8.

All of the operated animals leaked digestive fluid to the exterior via the mouth. Leaking frequently commenced as early as Day 2, and by Day 6 over 90% of the operated animals were losing fluid. Close inspection of these animals revealed that liquid escaping from the mouth quickly passed to the ventral region of the neck membrane and then to the dorsal

region. Some animals leaked more than others and in these individuals fluid could be detected along the lateral membranes of the abdomen.

Conclusions

Approximately one-third of the operated animals surviving beyond Day 6 exhibit a twofold or more increase in weight, develop a new cuticle, but fail in their attempt to moult to the adult stage. After the operation sensory impulses from the gut could still reach the brain and corpora cardiaca via the branches of the frontal connectives, and via the hypocerebral ganglion, NCC I pathway of STRONG (1966). On the motor side, the frontal connectives are obviously implicated in the processes of air intake and/or retention at ecdysis, and regurgitation.

CLARKE and LANGLEY (1963c) obtained a uniform growth response to the severance of both frontal connectives, the operated third instar nymphs maintaining a constant weight and failing to produce a new cuticle. Survival, however, was poor and this may have led to the elimination of potential growers. ROOME (1968), working with third instar Locusta nymphs and third and fourth instar Schistocerca gregaria nymphs, found that a few animals increased in weight and developed a new cuticle after the cutting of both frontal connectives. In Periplaneta americana the operation does not prevent normal crop emptying (DAVEY and TREHERNE, 1963), or growth and moulting by at least a few operated nymphs (ROOME, 1968). According to PENZLIN (1971), however, nymphal

Periplaneta are unable to moult successfully after the operation.

Experiment 5

The effect of cutting both frontal connectives (distal to branches) on growth and moulting

The experimental treatments were as follows:

(a)	Both frontal connectives cut (distal to branches)	24 animals
(b)	Operated controls	12 animals

Results

The operation was attended by an early high mortality, with 9 animals dead by Day 5. The rest survived for eleven or more days and can be divided into four groups according to their growth and moulting responses:

(i) Growth and attempted moulting

7 animals more than doubled their operation weights and died between Days 13 and 17 while trying to moult to adults. The attempted moult was made on average two days after its successful completion by the controls (Fig. 9). Table 5 shows that the mean maximum percentage weight increase of group (i) operated animals was significantly lower than that of the controls.

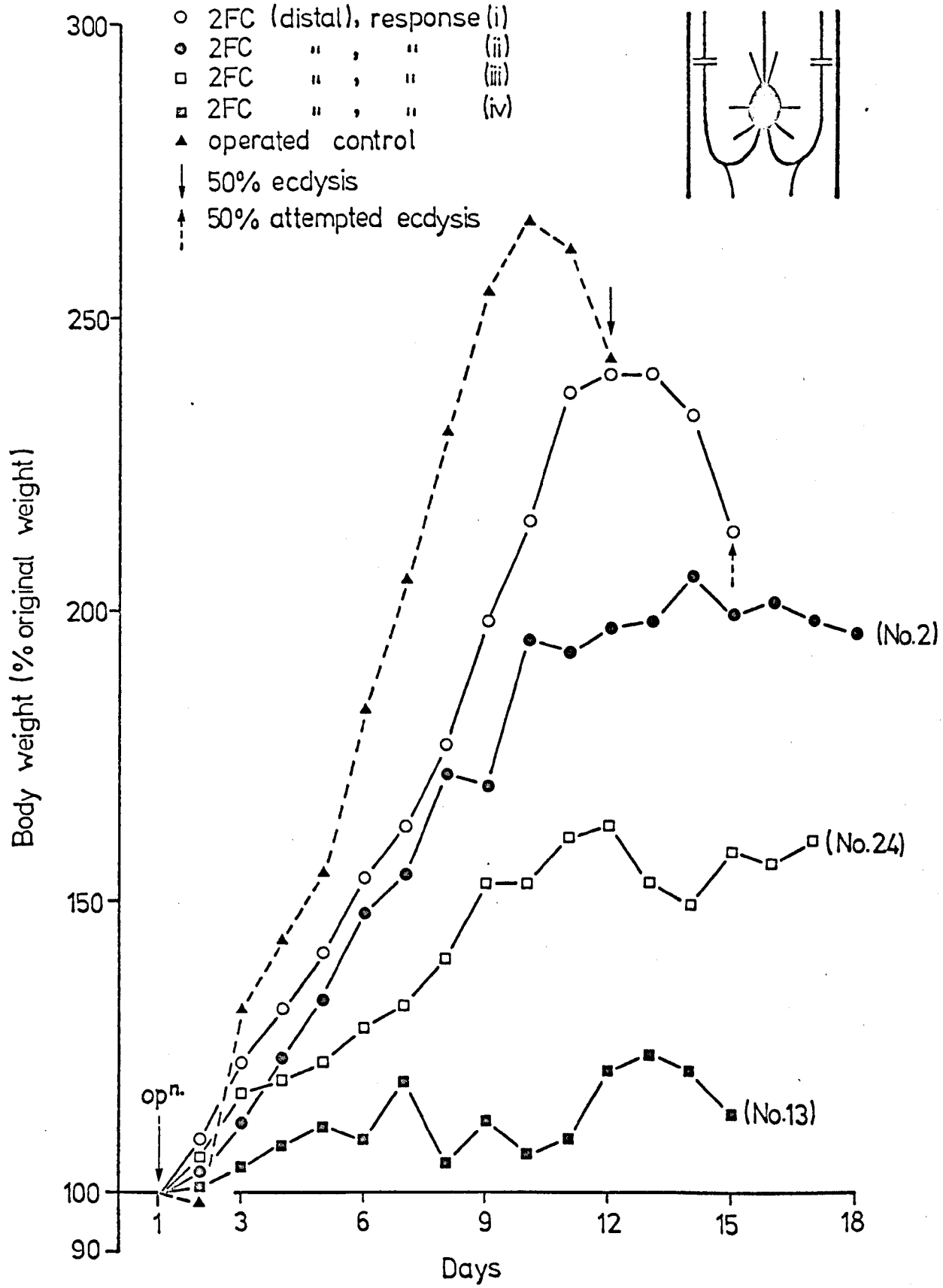

FIG 9. Growth after severance of two frontal connectives (distal).

TABLE 5. Comparison of the mean maximum percentage weight increases of group (i) operated animals and the controls

Treatment	Individuals	Mean	S.E.	t	'P'
2 FC's cut (distal) group (i)	7	132	10	3.50	0.01-0.001
Control	8	174	7		

Six operated animals were X-rayed over a period which covered the attempted moult and a sequence of radiographs from one representative individual (No. 5) is presented as Plates 34-37. It can be seen that the foregut contained only reduced amounts of air compared to the control animals at this time (Experiment 1), and that air continued to enter the hindgut after the operation. At autopsy food was always found in the midgut and ileum but not in the rest of the gut.

(ii) Growth and no attempted moulting

Operated animals Nos. 1 and 2 showed maximum weight increases of 105% and 106% respectively. The growth curve of No. 2 is presented in Fig. 9 where it can be seen that a period of growth during the first nine days is followed by a second period, also of nine days, during which a constant weight is maintained until death on Day 18. Neither animal developed a second cuticle.

PLATES 34-37. X-ray photographs, taken over a period covering the attempted moult, of a fifth instar locust subjected to severance of both frontal connectives (distal).

PLATE 34. 15½ hours before the attempted moult.

PLATE 35. 4¼ hours before the attempted moult.

PLATE 36. At the time of the attempted moulting.

PLATE 37. 8¼ hours after the attempted moult.

PLATES 34 – 37

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37

(iii) Reduced growth and no attempted moulting

Group (iii) is comprised of three animals. The growth curve of No. 24, reminiscent in general shape to that of No. 2, is presented in Fig. 9. No group (iii) animal ever developed a new cuticle.

(iv) Little or no growth and no attempted moulting

Two animals maintained a fairly constant weight after the operation, and the growth curve of one of these (No. 13) is presented in Fig. 9. Neither animal developed a new cuticle.

Of the fifteen animals surviving beyond Day 6, twelve leaked digestive fluid through the mouth, the first observation being recorded on Day 3 for two animals.

Conclusions

60% of the operated animals surviving beyond Day 6 undergo true growth and this is a clear indication that sensory impulses from the foregut can reach the brain and corpora cardiaca along routes other than those involving the frontal connectives. The hypocerebral ganglion, NCC I pathway of STRONG (1966a) is still open after the operation, and the possible involvement of other, as yet unidentified, nervous pathways should not be ruled out. The involvement of the frontal connectives in the processes of air intake and/or retention, and regurgitation is confirmed in this experiment.

Experiment 6

The effect of cutting the anterior, median and posterior pharyngeal nerves on growth and moulting

The experimental treatments were as follows:

(a)	APNs, MPNs, and PPNs cut	36 animals
(b)	Operated controls	15 animals

Results

4 operated animals were dead by Day 6. The rest can be divided into three groups according to their growth and moulting responses.

(i) Growth and attempted moulting

26 animals exhibited true growth and died between Days 13 and 20 while endeavouring to moult to adults. The attempted moult was made on average three days after its successful completion by the controls (Fig. 10). Table 6 shows that the mean maximum percentage weight gain of group (i) operated animals was significantly lower than that of the controls. The weight changes of two individual locusts, Nos. 11 and 36, are included in Fig. 10 to illustrate the extremes of growth encountered.

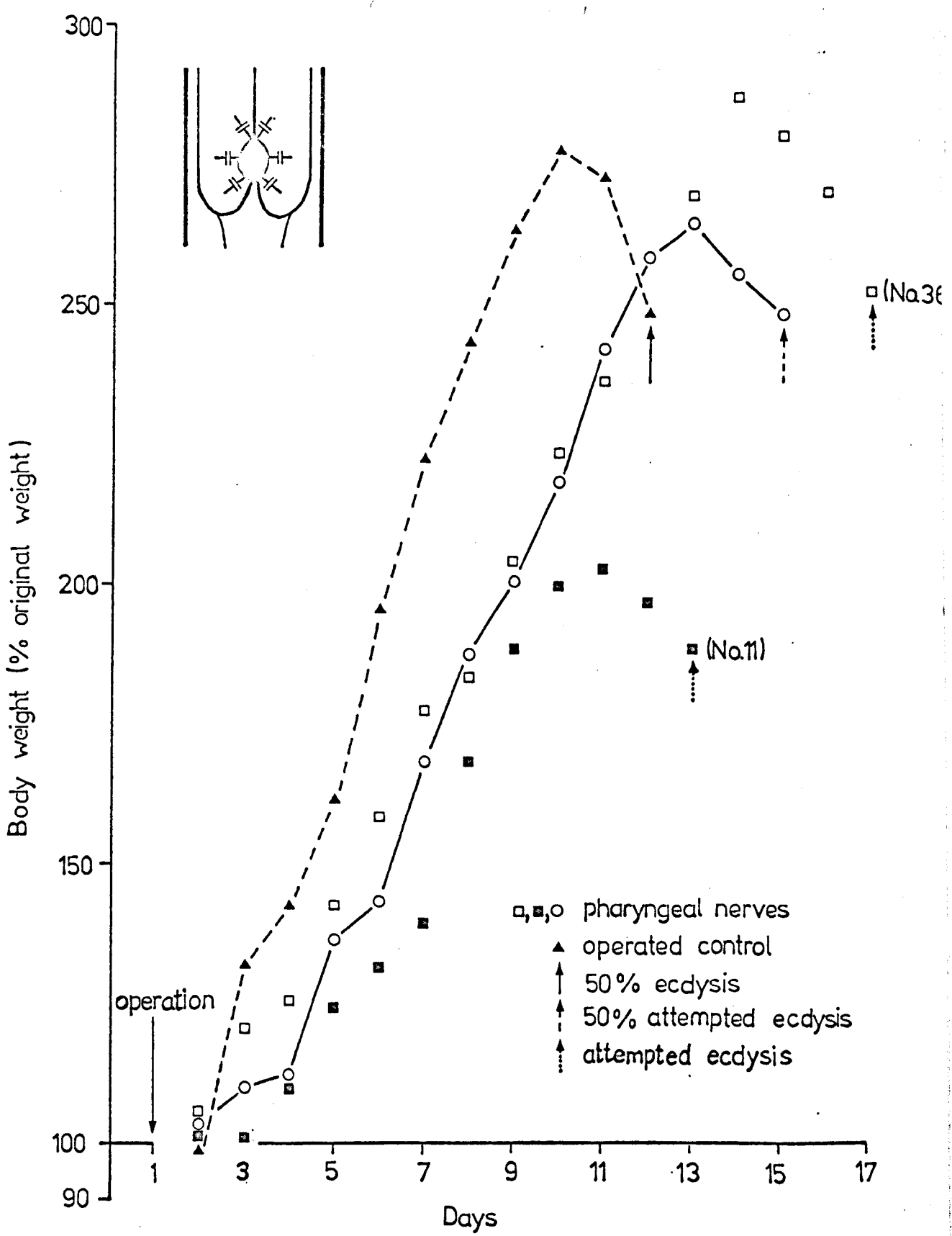

FIG 10. Growth after severance of anterior, median and posterior pharyngeal nerves.

TABLE 6. Comparison of the mean maximum percentage weight increases of group (i) operated animals and the controls

Treatment	Individuals	Mean	S.E.	t	'P'
APNs, MPNs and PPNs cut group (i)	26	158	4	3.60	0.001
Controls	12	176	3		

Ten operated locusts were X-rayed over a period covering the attempted moult, and a representative sequence of radiographs from one such animal (No. 16) is presented as Plates 38-41. It can be seen that reduced amounts of air are present in the foregut compared to the controls (Experiment 1), and that air continues to enter the hindgut. Post-mortem examination of group (i) animals indicated that food was usually absent from the crop, colon and rectum, but present in the midgut and ileum.

(ii) Growth and no attempted moulting

5 animals showed maximum body weight increases of between 118% and 166%. Two animals formed a new cuticle but died before making any attempt to moult. The other three animals failed to develop a new cuticle.

(iii) Reduced growth and no attempted moulting

Animal No. 38 increased its operation weight by a maximum of 92% before dying on Day 18 without ever forming a new cuticle.

PLATES 38-41. X-ray photographs, taken over a period covering the attempted moult, of a fifth instar locust subjected to severance of the anterior, median and posterior pharyngeal nerves.

PLATE 38. 11¼ hours before the attempted moult.

PLATE 39. 10 minutes before the attempted moult.

PLATE 40. At the time of attempted moulting.

PLATE 41. 12 hours after the attempted moult.

PLATES 38 – 41

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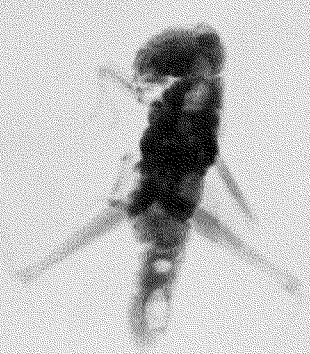

41

Twenty-two of the thirty-two operated animals surviving beyond Day 6 leaked digestive fluid, some individuals starting to lose fluid as early as Day 2.

Conclusions

97% of the operated animals surviving beyond Day 6 increase their operation weights by a factor of two or more. According to CLARKE and LANGLEY (1963c), severance of the pharyngeal nerves in third instar nymphs leads to an immediate cessation of growth. In Periplaneta the operation interferes with crop emptying (DAVEY and TREHERNE, 1963), but in Leucophaea maderae severance of all the nerves connected to the frontal ganglion, except the recurrent nerve, does not affect this process (ENGELMANN, 1968). The results of Experiment 6 compare favourably with those reported in Section I where it was shown that pharyngeal nerve severance in adult animals has no adverse effect upon growth during the first ten days post-operative.

After the operation sensory impulses from the foregut can still reach the brain and corpora cardiaca via the branches of the frontal connectives, and via the hypocerebral ganglion, NCC I pathway of STRONG (1966). On the motor side, the pharyngeal nerves obviously exert a controlling influence over the processes of air intake and/or retention at ecdysis, and regurgitation.

In two animals the operation was unsuccessful with, in each case, a single posterior pharyngeal nerve remaining intact. That both animals were able to moult to adults

renders it likely that a full complement of pharyngeal nerves is not a necessary requisite for successful moulting. This point is taken up in the next experiment.

Experiment 7

The effect of cutting the anterior or posterior pharyngeal nerves on growth and moulting

The experimental groups were as follows:

(a)	Anterior pharyngeal nerves cut	18 animals
(b)	Posterior pharyngeal nerves cut	16 animals
(c)	Operated controls	10 animals

Results

2 treatment (a) and 5 treatment (b) animals were dead by Day 4. Those surviving beyond this time can be divided into three groups according to their growth and moulting responses.

(i) Growth and successful moulting

9 treatment (a) and 8 treatment (b) animals underwent true growth and moulted successfully to adults. Moulting in treatment (b) animals was delayed on average by two days compared to the controls (Fig. 11). Table 7 shows that the mean maximum percentage weight increases of the operated and control groups were not significantly different.

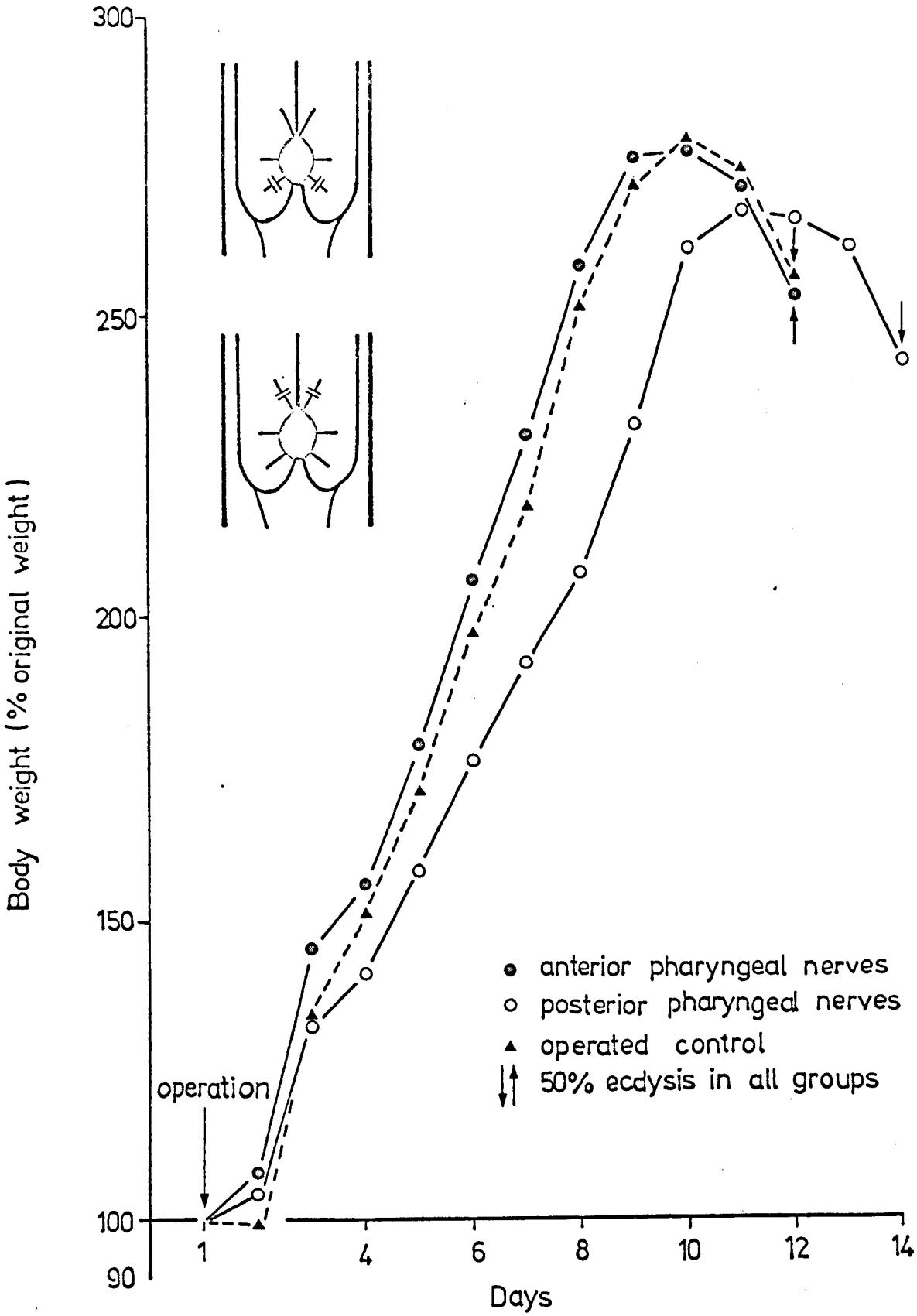

FIG 11. Growth after severance of anterior pharyngeal nerves, posterior pharyngeal nerves.

TABLE 7. Comparison of the mean maximum percentage weight increases of treatments (a) and (b) group (i) animals and the controls

Treatment	Individuals	Mean	S.E.		t	'P'
(a) APNs cut group (i)	9	179	5	(a):(b)	1.38	0.2-0.1
(b) PPNs cut group (i)	8	166	8	(a):(c)	-	
(c) Controls	9	179	3	(b):(c)	1.52	0.2-0.1

(ii) Growth and attempted moulting

6 treatment (a) and 2 treatment (b) animals underwent true growth and died between Days 12 and 16 while attempting to moult to adults. Table 8 shows that the mean maximum percentage weight increases of treatment (a) animals and the controls were not significantly different. The mean maximum percentage weight increase of the two treatment (b) animals was almost identical to that of treatment (a) animals and the controls.

TABLE 8. Comparison of the mean maximum percentage weight increases of treatments (a) and (b) group (ii) animals and the controls

Treatment	Individuals	Mean	S.E.		t	'P'
(a) APNs cut group (ii)	6	177	5			
(b) PPNs cut group (ii)	2	178	-	(a):(c)	0.34	0.8-0.7
(c) Controls	9	179	3			

(iii) Growth and no attempted moulting

Treatment (a) animal No. 3 and treatment (b) animal No. 3 exhibited maximum body weight increases of 186% and 101% respectively. Neither animal developed a second cuticle, the former dying on Day 16 and the latter on Day 10.

No treatment (a) or treatment (b) animal leaked digestive fluid at any time during the experimental period.

Conclusions

There exists a certain amount of individual variation in the moulting response to anterior or posterior pharyngeal nerve severance. The majority of animals in treatments (a) and (b) moult successfully to adults. In a few, however, both pairs of pharyngeal nerves need to be intact in order that successful moulting might take place. It is deduced that in these animals the moult fails because of a break-down in the neural mechanism co-ordinating the intake and/or retention of air at ecdysis.

Experiment 8

The effect of removing the frontal ganglion on growth and moulting

The experimental treatments were as follows:

(a)	Frontal ganglion removed	19 animals
(b)	Operated controls	8 animals

Results

The operated animals can be divided into four groups according to their growth and moulting responses.

(i) Growth and attempted moulting

Animals Nos. 3 and 15 showed maximum body weight increases of 104% and 118% respectively and died on Days 26 and 17 while attempting to moult to adults (Fig. 12). These weight increases were well below the mean maximum control increase of 175%.

Plate 42 is a radiograph of animal No. 3 taken at the time of attempted moulting. The moult failed because the insect was unable to empty its gut of food, a necessary preliminary to the swallowing of air. Despite being unable to split open the old cuticle, both group (i) operated animals made vigorous attempts to escape from it and this led to them assuming a shrunken appearance at death (Plate 29). Plates 44-47 are radiographs of four frontal ganglionectomised fourth instar nymphs (from Section III) which were taken at the time of their attempted moults. Food can again be seen to occupy most of the gut, although in three individuals air is detectable in the rectum. The above remarks concerning gut contents were confirmed on dissection.

(ii) Reduced growth and attempted moulting

Animal No. 7 increased its body weight by a maximum of 82% and died on Day 17 while attempting to moult. Plate 43 shows that this insect was unable to empty its gut of food at the time of moulting.

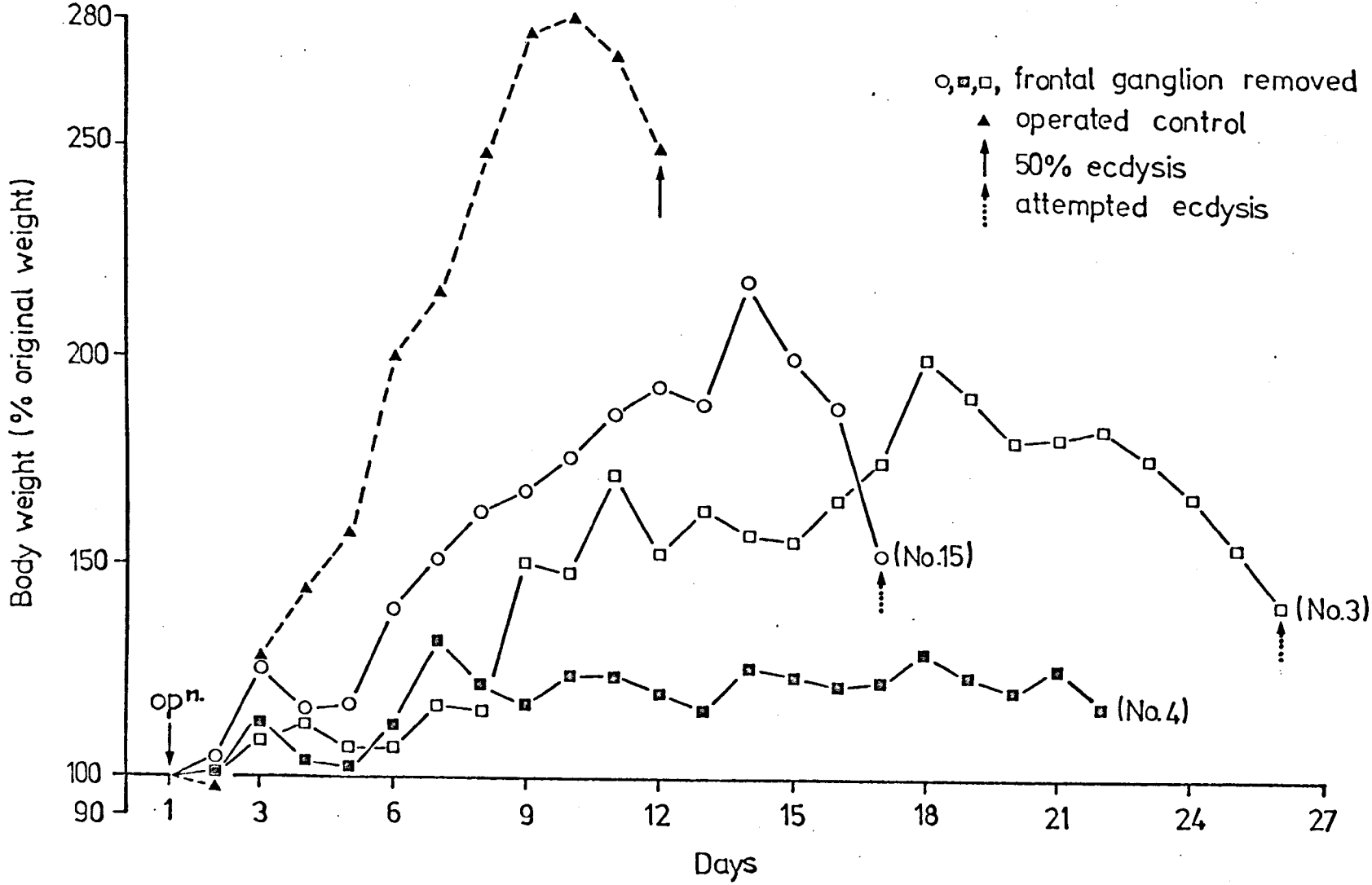

FIG 12. Growth after removal of the frontal ganglion.

PLATES 42, 43. X-ray photographs, taken at the time of attempted moulting, of two frontal ganglionectomised fifth instar locusts.

PLATES 44-47. X-ray photographs, taken at the time of attempted moulting, of four frontal ganglionectomised fourth instar locusts.

PLATES 42 - 47

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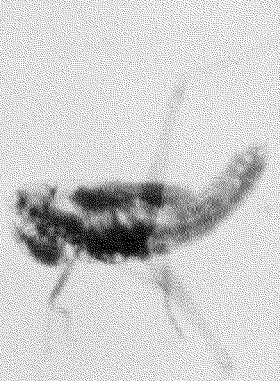

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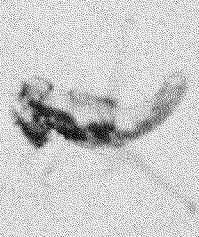

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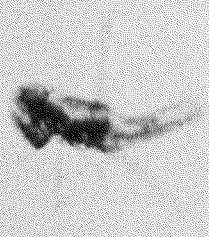

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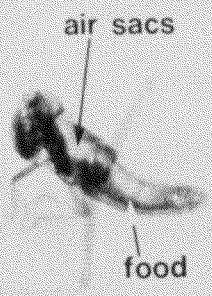

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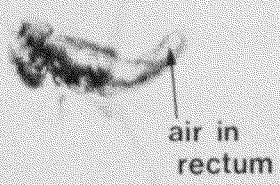


47

(iii) Reduced growth and no attempted moulting

3 animals showed maximum body weight increases of between 56% and 82% and failed to develop a new cuticle.

(iv) Little or no growth and no attempted moulting

13 animals maintained an approximately constant weight, and the growth curve of one such individual (No. 4), which survived for twice the normal instar length without ever forming a new cuticle, is presented in Fig. 12.

All of the operated animals leaked digestive fluid through the mouth, and in some leaking commenced as early as Day 2. The fluid dried on contact with the air, causing the head and thoracic regions in particular to become caked with the substance, so producing a picture consistent with that recorded by GILLOTT (1964) and ROOME (1968).

Conclusions

According to CLARKE and LANGLEY (1963b, c) frontal ganglionectomy in third instar nymphs causes every operated animal to maintain a constant weight. ROOME (1968), however, obtained essentially similar results to those described here, the majority of his frontal ganglionectomised third and fourth instar Locusta nymphs maintaining a constant weight, but a few showing fairly considerable weight increases after the operation. This author found that removal of the frontal ganglion in fourth instar Schistocerca nymphs caused them either to maintain a constant weight or to lose weight.

For continued growth to occur after the operation sensory impulses from the foregut must be reaching the brain

and corpora cardiaca along routes other than those involving the frontal ganglion. These may include the branches of the frontal connectives, and also the hypocerebral ganglion, NCC I route of STRONG (1966).

The frontal ganglion obviously plays an important role in co-ordinating emptying of the foregut at ecdysis. The rectum of a few fourth instar individuals was devoid of food and did, in fact, contain air. This is convincing proof that air does enter the hindgut through the anus at moulting. Its entry is most probably controlled by abdominal nerves running from the last abdominal ganglion to the rectum (ALBRECHT, 1953).

The progress of the moulting cycle is arrested in the majority of the operated animals and retarded by varying degrees in the rest. Animal No. 3, in attempting to moult on Day 26, took twice as long to reach this stage as did the controls. Animals Nos. 7 and 15, on the other hand, did not show such a lengthy delay, endeavouring to moult on Day 17 in each case.

Experiment 9

The effect of cutting (a) one labral nerve, and (b) both labral nerves on growth and moulting

The labral nerves, which probably contain both motor and sensory fibres, leave the tritocerebral ganglia and innervate the labrum and some of the dorsal muscles of the pharynx (SNODGRASS, 1926, 1935). In Periplaneta (WILLEY, 1961) the

labrum is also innervated by branches of the frontal connectives. The labral nerve in this insect has been shown by WILLEY to be of composite origin, coming partly from cells within the tritocerebrum and sub-oesophageal ganglion, and partly from fibre tracts of the protocerebrum. In Locusta, as in Periplaneta, each labral nerve fuses with its fellow frontal connective to form a short labro-frontal nerve root which then enters the tritocerebrum. Thus, although not forming part of the stomatogastric nervous system, the labral nerves are intimately associated with one of its components. In Experiment 9 the effects upon growth and moulting produced by severing one or both labral nerves are tested.

The experimental treatments were as follows:

(a)	One labral nerve cut	10 animals
(b)	Both labral nerves cut	24 animals
(c)	Operated controls	10 animals

Results

The severance of one labral nerve had no adverse effect upon the growth and moulting cycle (Fig. 13, Table 9).

An early high mortality attended severance of both labral nerves and eleven of the operated animals were dead by Day 6. Those surviving beyond this time can be divided into four groups according to their growth and moulting responses.

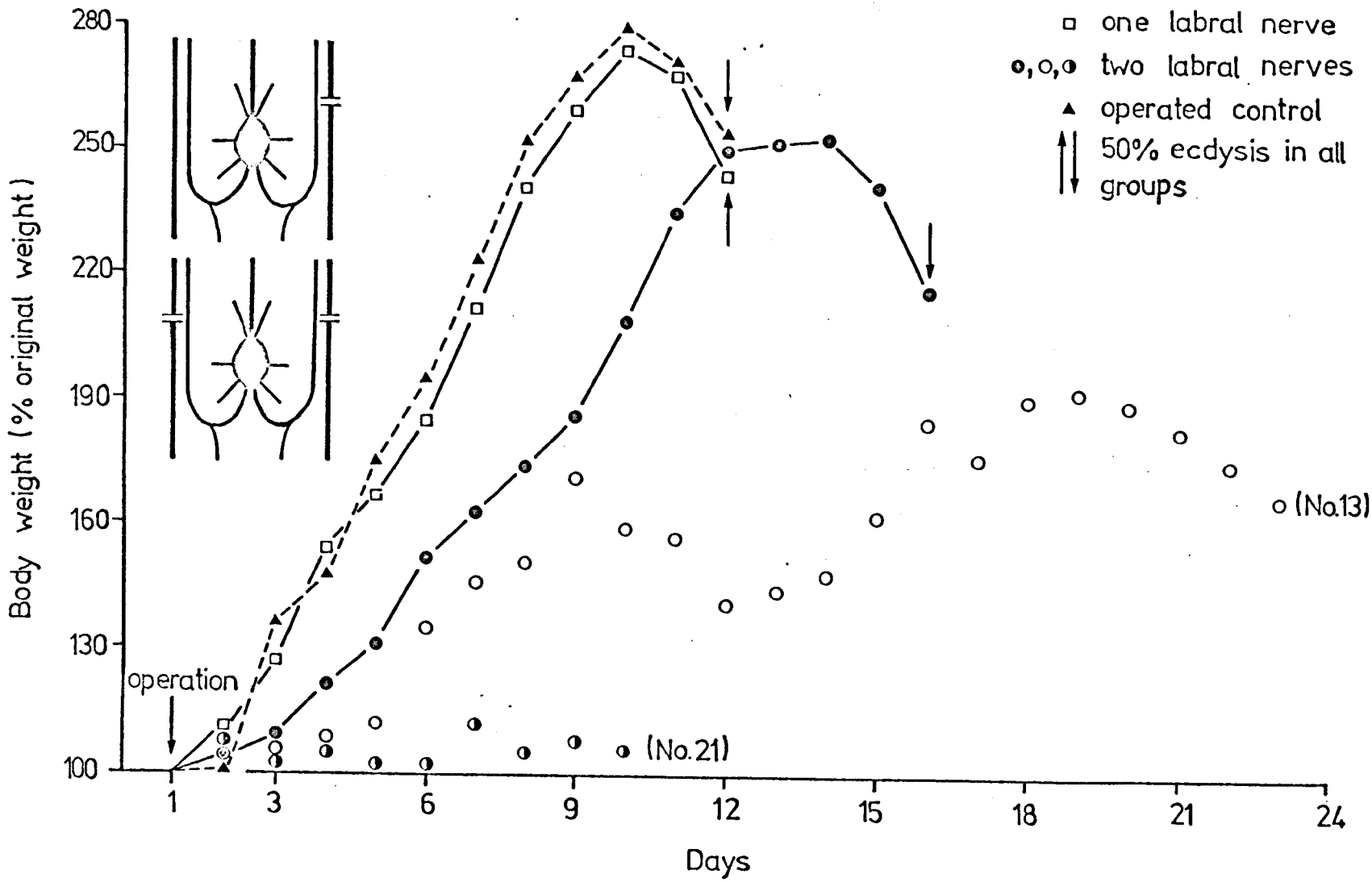


FIG 13. Growth after severance of one labral nerve, two labral nerves.

TABLE 9. Comparison of the mean maximum percentage weight increases of treatment (a), treatment (b) group (i), and treatment (c) animals

Treatment	Individuals	Mean	S.E.		t	'P'
(a) 1 LN cut	10	181	6	(a):(b)	3.04	0.01-0.001
(b) 2 LNs cut group (i)	6	153	7	(a):(c)	0.60	0.6-0.5
(c) Controls	10	177	3	(b):(c)	3.15	0.01-0.001

(i) Growth and successful moulting

6 animals underwent true growth and moulted to adults, though an average four days later than the controls (Fig. 13). Table 9 shows that the mean maximum percentage weight increase of these animals was significantly lower than that of treatment (a) animals and the controls.

(ii) Growth and no moulting

Animals Nos. 1 and 23 exhibited maximum body weight increases of 126% and 121% respectively. Both formed a new cuticle but died without ever attempting to moult.

(iii) Reduced growth and no moulting

3 animals showed maximum body weight increases that were 50-100% above their operation weights. No animal in this group ever developed a new cuticle, although No. 13 survived for more than twice the normal instar length (Fig. 13).

(iv) Little or no growth and no moulting

2 animals maintained an approximately constant weight after the operation, one for eight and the other for nine days. The weight changes of the latter animal are presented in Fig. 13. Neither animal ever developed a new cuticle.

Grass accumulated in the preoral food cavity of five treatment (b) animals (Table IX of the Appendix) forcing the labrum upwards and away from its normal resting position. Labrum displacement was a temporary phenomenon and neither growth nor moulting were prevented by it. No treatment (a) or treatment (b) animal leaked digestive fluid at any time during the experiment.

Conclusions

True growth can occur in the absence of sensory information normally carried by the labral nerves. It is assumed here that the labral nerves in Locusta normally carry sensory information from the A_1, A_2, and A_3 receptors of the clypeolabrum (THOMAS, 1966). HASKELL and MORDUE (1969) have shown that cautery of the $A_1 + A_2$ receptors, or cautery of the A_3 receptors, does not seriously affect feeding behaviour. After labral nerve severance the central nervous system can still receive phagostimulatory input from the H receptors on the hypopharynx, and from the dome receptors on the labral and maxillary palps, which regions are innervated by nerves from the sub-oesophageal ganglion (ALBRECHT, 1953).

On the motor side, the cutting of both labral nerves interferes with the role of the labrum in assisting entry of food into the mouth. The accumulation of food in the preoral food cavity is, however, only a temporary phenomenon, and in the absence of the labral nerves, the branches of the frontal connectives may control movement of the labrum.

Summary and Conclusions to Section II

1. A summary of the results of the various operations performed in this section is presented in Table 10.

2. True growth (minimum twofold increase in body weight) can occur after the severance of both frontal connectives, after the severance of the anterior, median and posterior pharyngeal nerves, and after the removal of the frontal ganglion. These results should be compared with those of CLARKE and LANGLEY (1963b, c) who state that the above operations lead to a complete cessation of growth in third instar nymphs.

3. Sensory information from the foregut can reach the brain and corpora cardiaca along nervous pathways which do not involve the frontal ganglion. These may include the branches of the frontal connectives, the hypocerebral ganglion ⟶ NCC I pathway of STRONG (1966), and other as yet unidentified routes.

TABLE 10 A summary of the results of the various operations performed in Section II

Exp. No.	Treatment	No. operated	No. living beyond Day 6	% Animals surviving beyond Day 6 showing							
				Weight increase (%)			Digestive fluid loss	Labrum displacement	New cuticle formation	Moulting	
				0-50	50-100	100				Successful	Unsuccessful
2	RN cut	14	7*	0	0	100	0	0	100	100	0
3	1 FC cut (prox.)	16	14	0	0	100	0	0	100	100	0
	1 FC cut (dist.)	14	12	0	0	100	0	0	100	100	0
4	2 FCs cut (prox.)	47	39	36	23	41	100	0	46	0	44
5	2 FCs cut (dist.)	24	15	13	27	60	80	0	47	0	47
6	All PNs cut	36	32	0	3	97	69	0	88	0	81
7	APNs cut	18	16	0	0	100	0	0	94	56	38
	PPNs cut	16	11	0	0	100	0	0	91	73	18
8	FG removed	19	19	68	21	11	100	0	16	0	16
9	1 LN cut	10	10	0	0	100	0	0	100	100	0
	2 LNs cut	24	13	15	23	62	0	38	62	46	0

* ANIMALS NOT EXHIBITING HYPERPHAGIC-TYPE RESPONSE

4. The frontal ganglion co-ordinates emptying of the foregut at ecdysis.

5. A motor pathway involving the frontal connectives, the frontal ganglion, and the pharyngeal nerves is implicated in the control of air intake and/or retention at ecdysis. This same pathway also controls regurgitation.

6. The labral nerves are not involved in the control of growth and moulting.

SECTION III

THE EFFECT OF FRONTAL GANGLIONECTOMY ON FOOD PASSAGE AS MEASURED BY FAECES PRODUCTION

There is much evidence to suggest that frontal ganglionectomy interferes with the movement of food through the gut. In immature adult female Schistocerca gregaria removal of the frontal ganglion inhibits emptying of the foregut (HIGHNAM et al., 1966); the amount of food passed through the gut of these animals, as measured by faeces production, is greatly reduced (HILL et al., 1966). The operation has a similar effect on crop emptying and faeces production in immature adult female Melanoplus differentialis (GILLOTT et al., 1970; DOGRA and EWEN, 1971) and adult male and female Gryllus bimaculatus (ROUSSEL, 1966).

On the other hand, CLARKE and LANGLEY (1963b) and CLARKE and GILLOTT (1965) report that food intake and defaecation proceed normally in third instar Locusta nymphs after removal of the frontal ganglion. Immature adult female Necrophorus vespillo were observed by ROUSSEL (1966) to feed normally and to show an increase in body weight after frontal ganglionectomy. However, with the exception of HILL et al. (1966), none of the above authors include any quantitative measurements of feeding activity.

ROOME (1968) has shown that frontal ganglionectomy in third and fourth instar Locusta nymphs leads to a significant

drop in food consumption and faeces production in the majority of operated animals. This effect is related to an observed accumulation of food in the crop at autopsy and to an absence of any real growth. In a few operated individuals, however, positive growth is identified and is associated with an increase in faeces production.

The results of Experiment 8 (Section II) demonstrate the variable effect of frontal ganglionectomy upon the growth and moulting cycle in fifth instars. Thus while the majority of operated animals maintain a constant weight and fail to develop a new cuticle, a few grow, form a new cuticle and then die while attempting to moult to the adult stage. Casual observation of faeces production indicated that the former group of animals were passing more food through the gut than the latter group

In Section III the various growth responses to frontal ganglionectomy are related to food passage through the gut, as measured by faeces production. The frontal ganglion was removed, without the addition of any Ringer solution, from newly moulted fourth and fifth instar Locusta nymphs of both sexes. Operated and control animals were weighed daily and their faeces collected, dried at 100°C for 24 hr and then weighed. Operative technique and post-operative care were as described in Chapter II. ROOME (1968) has investigated faeces production in short-lived (dead before Day 10) frontal ganglionectomised Locusta nymphs. Therefore only those operated animals surviving for longer than ten days are considered here.

Fifth Instars

Fifty-four newly moulted fifth instar nymphs of both sexes were sampled from a stock cage; the frontal ganglion was removed from thirty-eight animals (16 m, 22 f) and sixteen animals (7 m, 9 f) served as controls.

The controls had an instar length of 12 to 13 days (Figs. 17 and 18), compared to the 10 days of normal unoperated animals. The delay in moulting can be attributed to the post-operative treatment of the controls. Control female locusts passed more food through the gut per day than control males and were correspondingly larger and heavier (Table 21). Faeces production in both sexes reached a peak during the middle period of the instar (Figs. 19 and 20), a result agreeing with that of DAVEY (1954) for unoperated fifth instar Schistocerca nymphs.

Eight operated animals died before Day 10 and were discarded. The rest survived for longer than ten days and are divided into four groups according to their growth and moulting responses:

(a) Growth (at least 100% increase in weight) and attempted moulting

Thirteen animals grew, though at a reduced rate when compared to the controls (Figs. 17 and 18), and developed a new cuticle underneath the old one. They all died, between Days 14 and 22, while attempting to moult to the adult stage. The condition of the gut of these animals at autopsy is

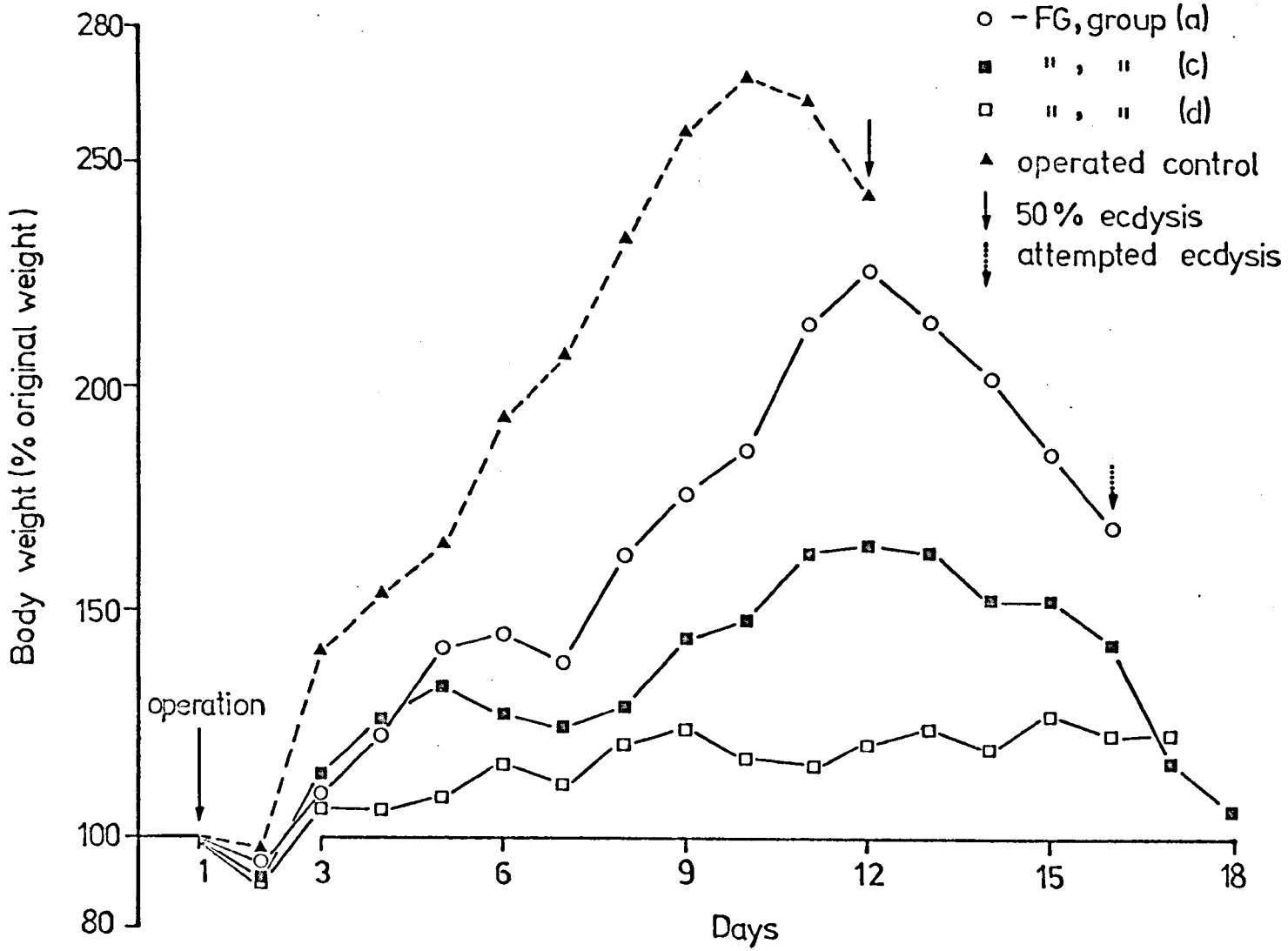


FIG 17. Growth after removal of the frontal ganglion (5th instar, ♂).

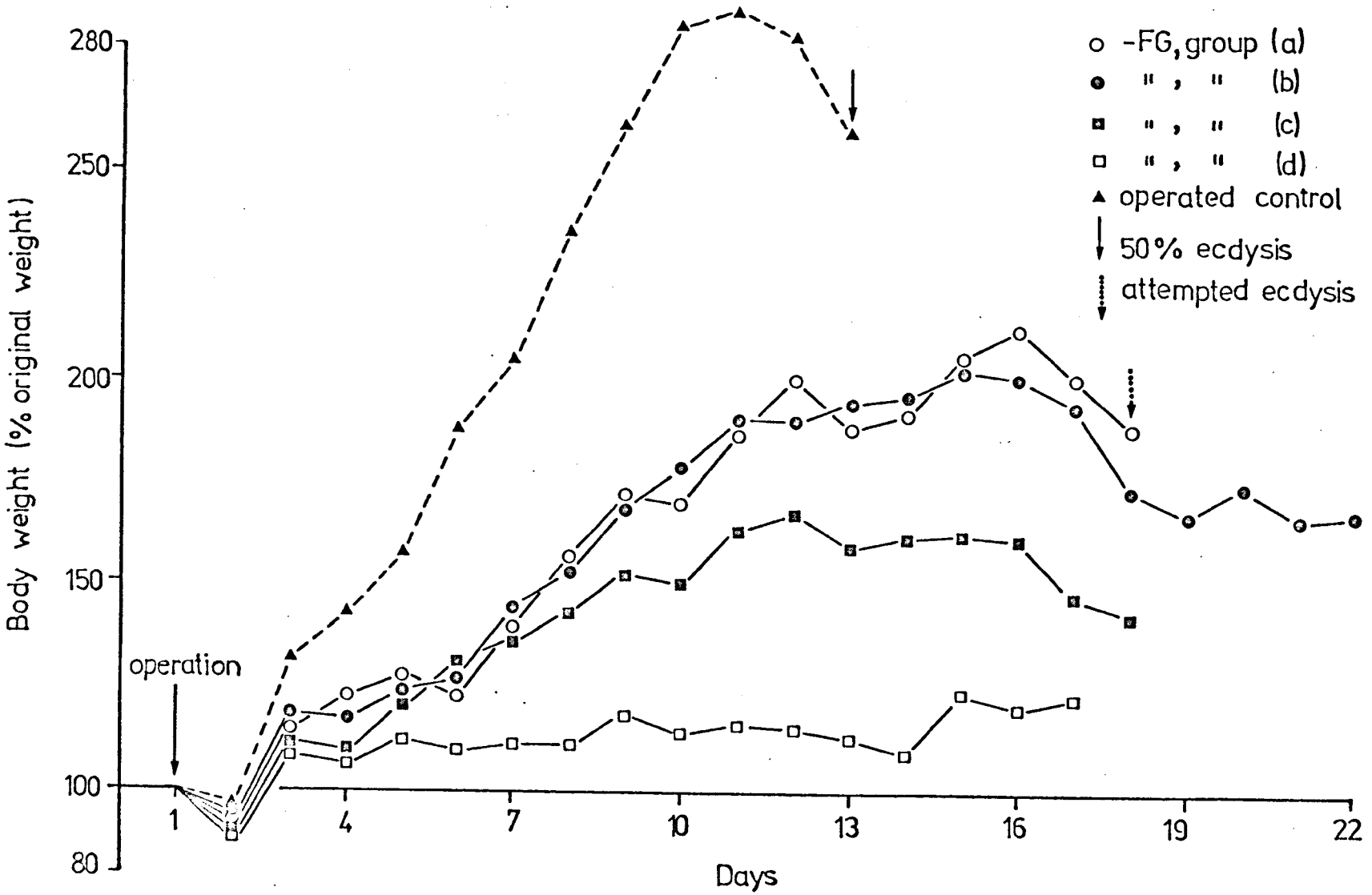


FIG 18. Growth after removal of the frontal ganglion (5th instar, ♀).

TABLE 21 The relationship between faeces production and weight increase in fourth and fifth instar males and females. (Summary of Tables X and XI of the Appendix)

Fifth Instar

	Male			Female		
	No. of animals in group	Mean max % body weight gain	Mean daily faeces prodn. (mg/day)	No. of animals in group	Mean max % body weight gain	Mean daily faeces prodn. (mg/day)
Controls	5	169	61	9	189	85
Operated animals:						
(a) Growth and AM	7	128	48	6	119	51
(b) Growth and no AM	-	-	-	2	105	38
(c) Reduced growth and no AM	4	70	31	4	70	32
(d) Little or no growth and no AM	2	37	17	5	27	22

Fourth Instar

Controls	5	148	31	6	180	42
Operated animals:						
(a) Growth and AM	1	130	23	1	121	24
(b) Reduced growth and AM	1	57	15	2	71	17
(c) Reduced growth and no AM	3	62	17	3	64	18
(d) Little or no growth and no AM	2	29	12	4	31	13

AM, Attempted moulting.
Operation body weight = 0%

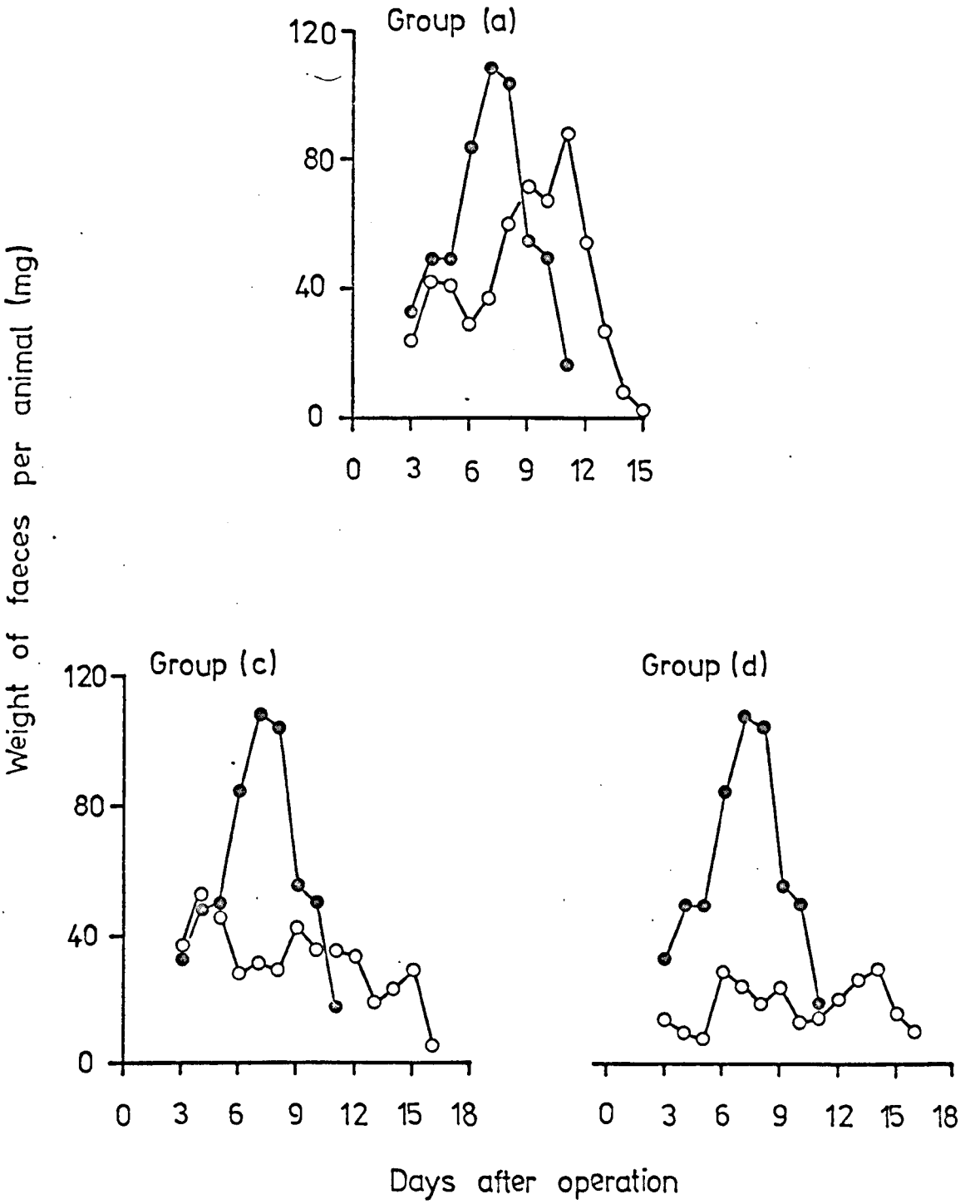


FIG 19. The weight of faeces produced per animal per day following frontal ganglionectomy of fifth instar males.
o— frontal ganglionectomized animals; ●— control animals.

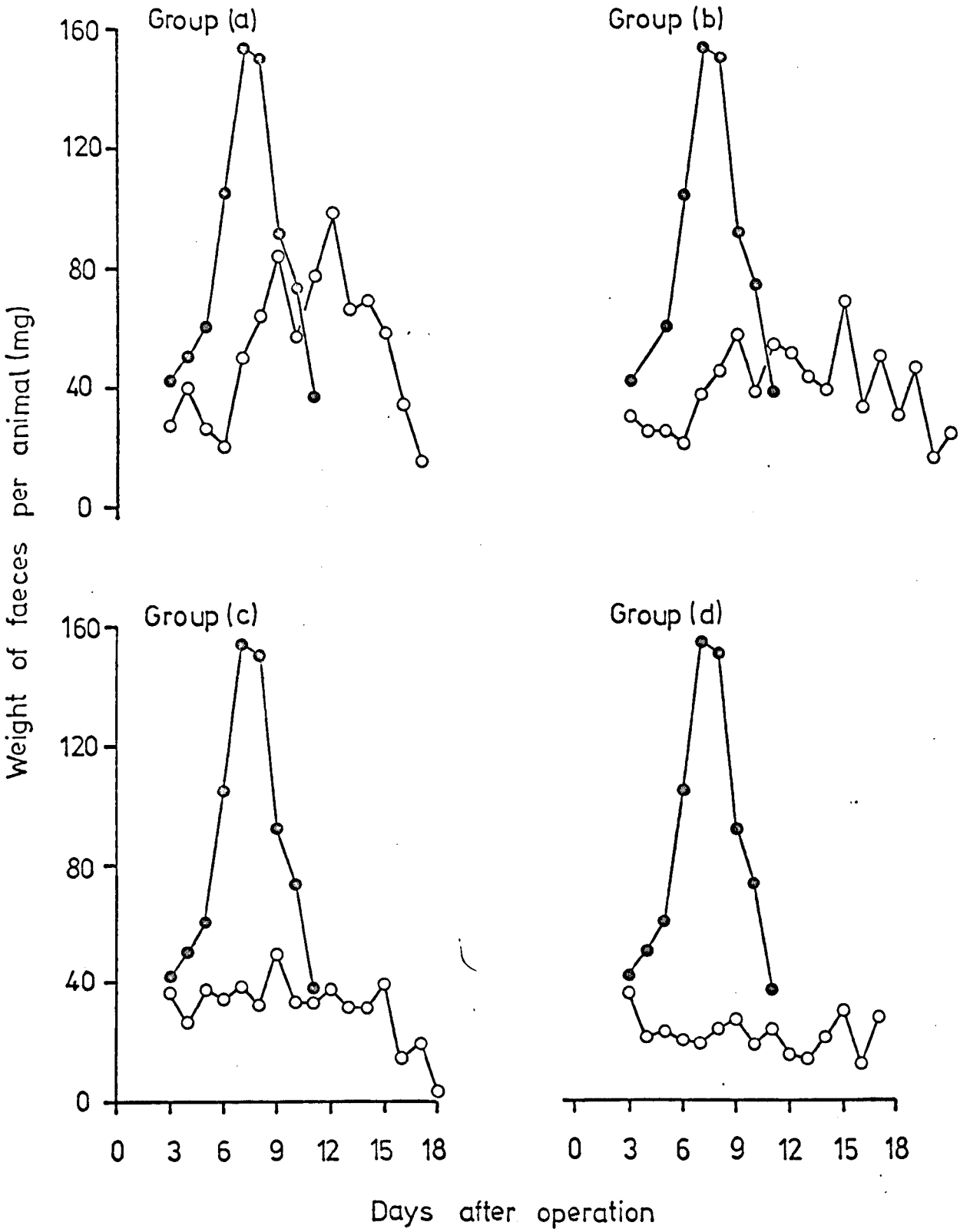


FIG 20. The weight of faeces produced per animal per day following frontal ganglionectomy of fifth instar females.
o— frontal ganglionectomized animals; •— control animals.

summarized in Table 22. It is clear that frontal ganglionectomy prevents normal emptying of the foregut prior to swallowing of air at ecdysis. Air was, however, observed in the rectum of a few individuals.

Group (a) operated animals of both sexes passed less food through the gut per day than the controls and their body weight increases were correspondingly lower (Table 21). The operated peak in faeces production was lower than the control peak and appeared later (Figs. 19 and 20). In general it was found that the longer the operated instar period, the later was the appearance of the peak in faeces production. Thus operated female No. 1 died trying to moult on Day 22 and showed a faeces peak on Day 18, while operated Male No. 13 attempted to moult on Day 14 and exhibited a faeces peak on Day 9, just one day later than the control peak (Table X of the Appendix).

(b) Growth (at least 100% increase in weight) and no attempted moulting

Two females grew, but at a reduced rate compared to the controls (Fig. 18), and died without forming a new cuticle. At autopsy the foregut, midgut and ileum of female No. 15 (survived for sixteen days) was packed full of grass, while the gut of female No. 14 (survived for twenty-two days) contained more normal amounts of food (Table 22). Despite the distended state of its gut, female No. 15 expelled a greater weight of faeces per day than female No. 14 (Table X of the Appendix). Daily faeces production and body weight increase were both reduced in comparison to group (a) operated females (Table 21, Fig. 20).

TABLE 22 Food content in operated fifth instar animals at autopsy

Group (a) Growth (at least 100% increase in weight) and attempted moulting

Animal No.	Food content of gut				
	Foregut	Midgut	Ileum	Hindgut Colon	Rectum
1	++	++	+	+	Air
2	++	++	-	-	-
3	++	+	-	+ (Rm)	+ (Rm)
4	+++	+	-	-	+ (Rm)
5	++	++	+	-	+
6	+++	++	+	+	Air
7	++	++	-	-	-
8	+++	++	-	-	+ (Rm)
9	++	+	-	-	+
10	++	++	-	-	+ (Rm)
11	++	++	+	+	-
12	+++	++	-	-	-
13	++	++	-	-	-

Group (b) Growth (at least 100% increase in weight) and no attempted moulting

14	+++	+++	+	-	++
15	+++++	+++++	++++	-	++

TABLE 22 (continued)

Group (c) Reduced growth (50-100% increase in weight) and no attempted moulting

16	+++++	+++++	+++++	-	-
17	+++	++	-	-	++
18	+++++	++	-	-	+ (Rm)
19	+++++	++++	+	-	++ (Rm)
20	+++++	+++	+++	-	+ (Rm)
21	+++++	+++++	+++++	-	-
22	++++	++++	-	-	+++ (Rm)
23	+++	++	+	-	-

Group (d) Little or no growth (0-50% increase in weight) and no attempted moulting

24	++++	+++	-	-	++ (Rm)
25	+++++	++++	+++	-	+
26	++++	+	-	-	+ (Rm)
27	++++	++	-	-	+
28	+++++	+	-	-	+ (Rm)
29	++++	+++	-	-	++
30	++++	+	-	-	++ (Rm)

Scoring of gut contents:

+, Very little food +++++, Gut distended with food;

-, Food absent; Rm, Red material.

(c) Reduced growth (50-100% increase in weight) and no attempted moulting

Four males and four females exhibited weight increases that were 50-100% above their operation weights (Figs. 17 and 18). None of them ever developed a new cuticle. The foregut of the majority of animals was packed full of grass at autopsy and in some cases the midgut and ileum were also distended with food (Table 22). The reduced growth rate of group (c) operated animals was associated with a drop in the daily production of faeces (Table 21, Figs. 19 and 20).

(d) Little or no growth (0-50% increase in weight) and no attempted moulting

Two males and five females maintained an approximately constant weight after removal of the frontal ganglion (Figs. 17 and 18) and did not develop a new cuticle. The foregut of every individual was found to be distended with undigested food at autopsy (Table 22). Group (d) operated animals maintained a constant, low level of faeces production throughout their lives (Figs. 19 and 20), and this was associated with a low weight increase (Table 21). The peak in faeces production, so obvious in control and group (a) operated animals, was completely suppressed in groups (c) and (d) operated animals.

Fourth Instars

Forty-two newly moulted fourth instar nymphs of both sexes were sampled from a stock cage; thirty animals (15 m, 15 f) had the frontal ganglion removed and twelve (6 m, 6 f) served as controls.

The controls had an instar length of 9 days (Figs. 21 and 22) compared to the 7 days of normal unoperated animals. As in fifth instars the delay in moulting can be attributed to the post-operative treatment of the controls. Control females passed more food through the gut per day than control males and were correspondingly larger and heavier (Table 21). A single, mid-instar peak in faeces production is recorded for operated control fourth instar Locusta females (ROOME, 1968) and for unoperated fourth instar Schistocerca nymphs (DAVEY, 1954). Two peaks in faeces production were manifest in the control males of the present experiment and although a single peak was evident in the control females it did not appear until two days before the moult (Figs. 23 and 24).

Thirteen operated animals died before Day 10 and were discarded. The rest survived for longer than ten days and are divided into four groups according to their growth and moulting responses:

(a) Growth (at least 100% increase in weight) and attempted moulting

One male (No. 2) and one female (No. 1) exhibited weight increases in excess of 100% of their original weights,

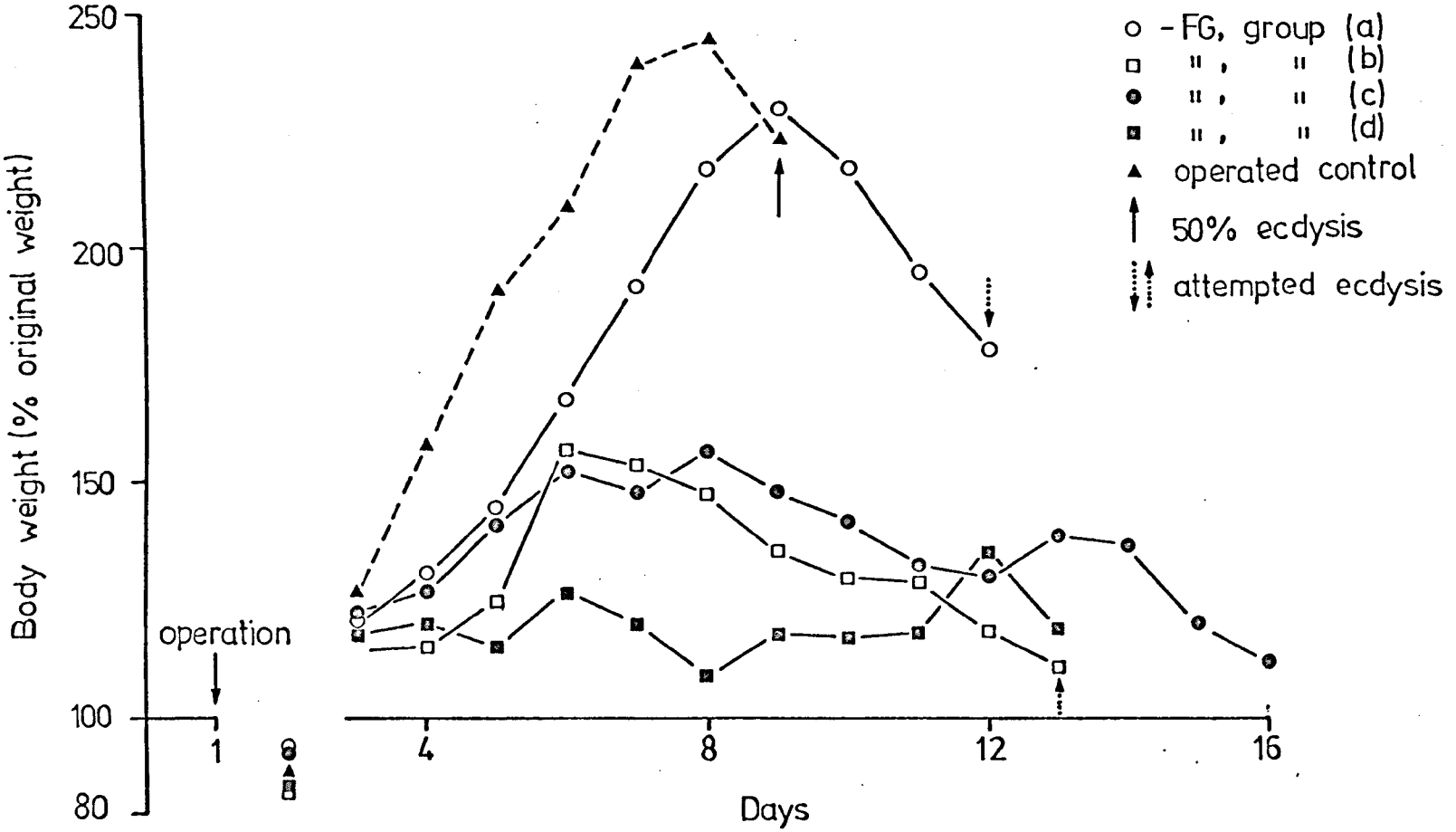


FIG 21. Growth after removal of the frontal ganglion (4th instar, ♂).

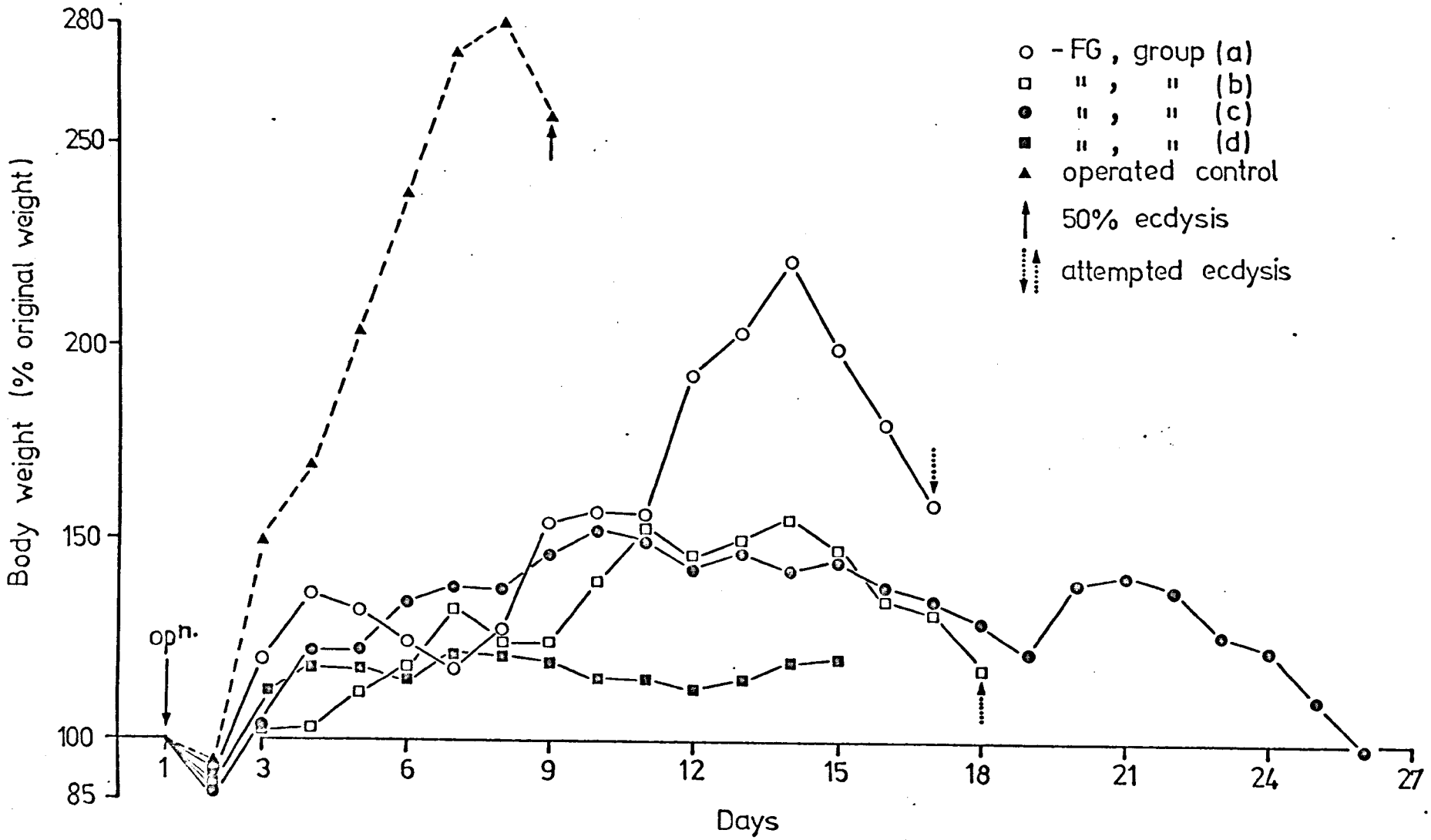


FIG 22. Growth after removal of the frontal ganglion (4^{th} instar, ♀).

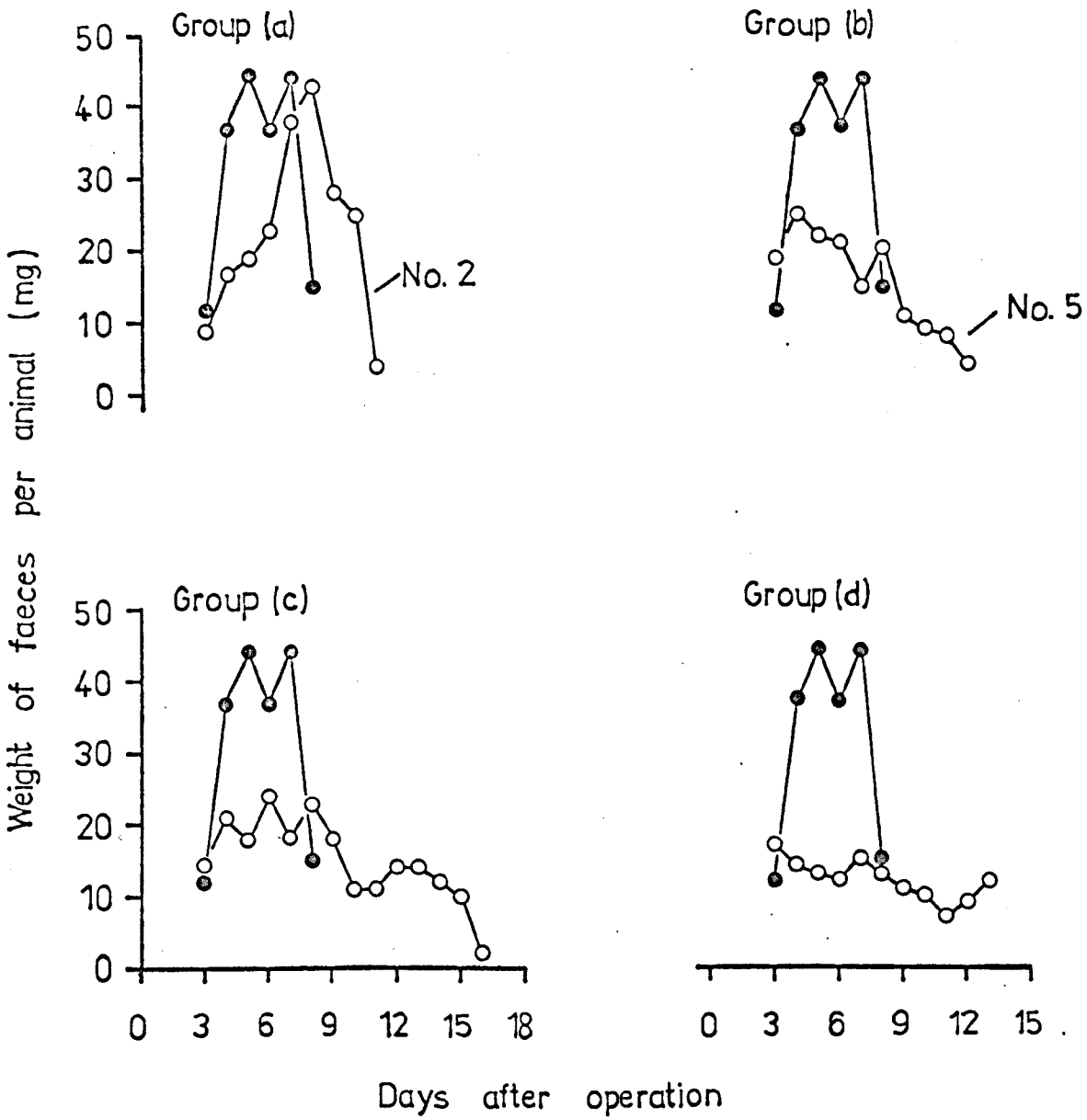


Fig 23. The weight of faeces produced per animal per day following frontal ganglionectomy of fourth instar males. o— frontal ganglionectomized animals; ●— control animals.

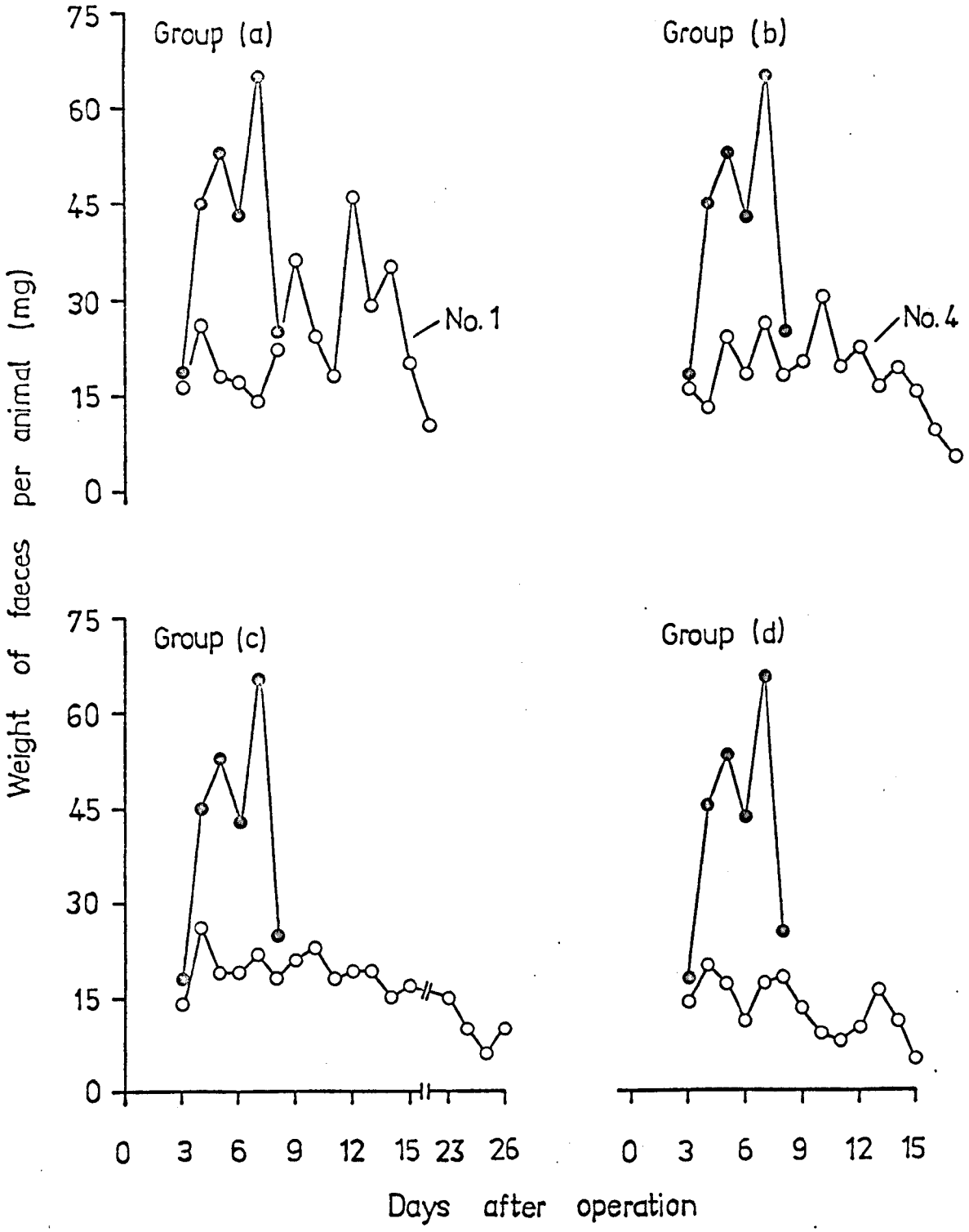


Fig 24. The weight of faeces produced per animal per day following frontal ganglionectomy of fourth instar females. o—frontal ganglionectomized animals, ●— control animals.

developed a new cuticle, and died while trying to moult to fifth instars on Days 12 and 17 respectively (Figs. 21 and 22). Moderate amounts of food were present in the foregut and midgut of both animals at autopsy (Table 23). Less food was present in the hindgut, and air was detected in the rectum of one animal (No. 2). Death was a result of the operated animals being unable to empty the foregut of food prior to swallowing air at ecdysis. The mean daily level of faeces production in both sexes was reduced in comparison to the controls (Table 21). Despite this the peak in faeces production of operated male No. 2 was the same height as the mean control male peaks (Fig. 23).

(b) Reduced growth (50-100% increase in weight) and attempted moulting

One male (No. 5) and two females (Nos. 3 and 4) showed maximum weight increases that were 50-100% above their operation weights. All three developed a new cuticle but failed in their attempts to moult to fifth instars. The growth curves of male No. 5 and female No. 5 are presented in Figs. 21 and 22 and it can be seen that they attempted to moult at weights which were only 11% and 17% respectively above their operation weights. At autopsy the foregut and midgut of group (b) operated animals contained moderate amounts of food which was otherwise absent from the rest of the gut (Table 23). Air was detected in the rectum of two animals (Nos. 3 and 4). Animals in group (b), like those in group (a), died because they were unable to empty the foregut of food at the time of the moult. Daily faeces production

TABLE 23 Food content in operated fourth instar animals at autopsy

Group (a) Growth (at least 100% increase in weight) and attempted moulting

Animal No.	Food content of gut				
	Foregut	Midgut	Ileum	Hindgut Colon	Rectum
1	+++	+++	++	+	-
2	+++	+++	+	-	Air

Group (b) Reduced growth (50-100% increase in weight) and attempted moulting

3	+++	+++	-	-	Air
4	+++	+++	-	-	Air
5	+++	+++	-	-	-

Group (c) Reduced growth (50-100% increase in weight) and no attempted moulting

6	++++	++	++	-	+ (Rm)
7	+++	+++	-	-	-
8	+++	++	++	+	+++
9	+++++	++	++	-	+ (Rm)
10	++++	+++	-	-	+
11	+++++	++++	++	-	+

TABLE 23 (continued)

Group (d)	Little or no growth (0-50% increase in weight) and no attempted moulting				
12	++++	+	-	-	+ (Rm)
13	+++++	+++	+	-	++
14	++++	+++	-	-	+
15	++++	++	-	-	-
16	++++	+	+	-	+ (Rm)
17	++++	++	-	-	-

Scoring of gut contents:

+, Very little food +++++, Gut distended with food;

-, Food absent; Rm, Red material.

and body weight increase in both sexes were reduced in comparison to group (a) operated animals (Table 21, Figs. 23 and 24).

(c) Reduced growth (50-100% increase in weight) and no attempted moulting

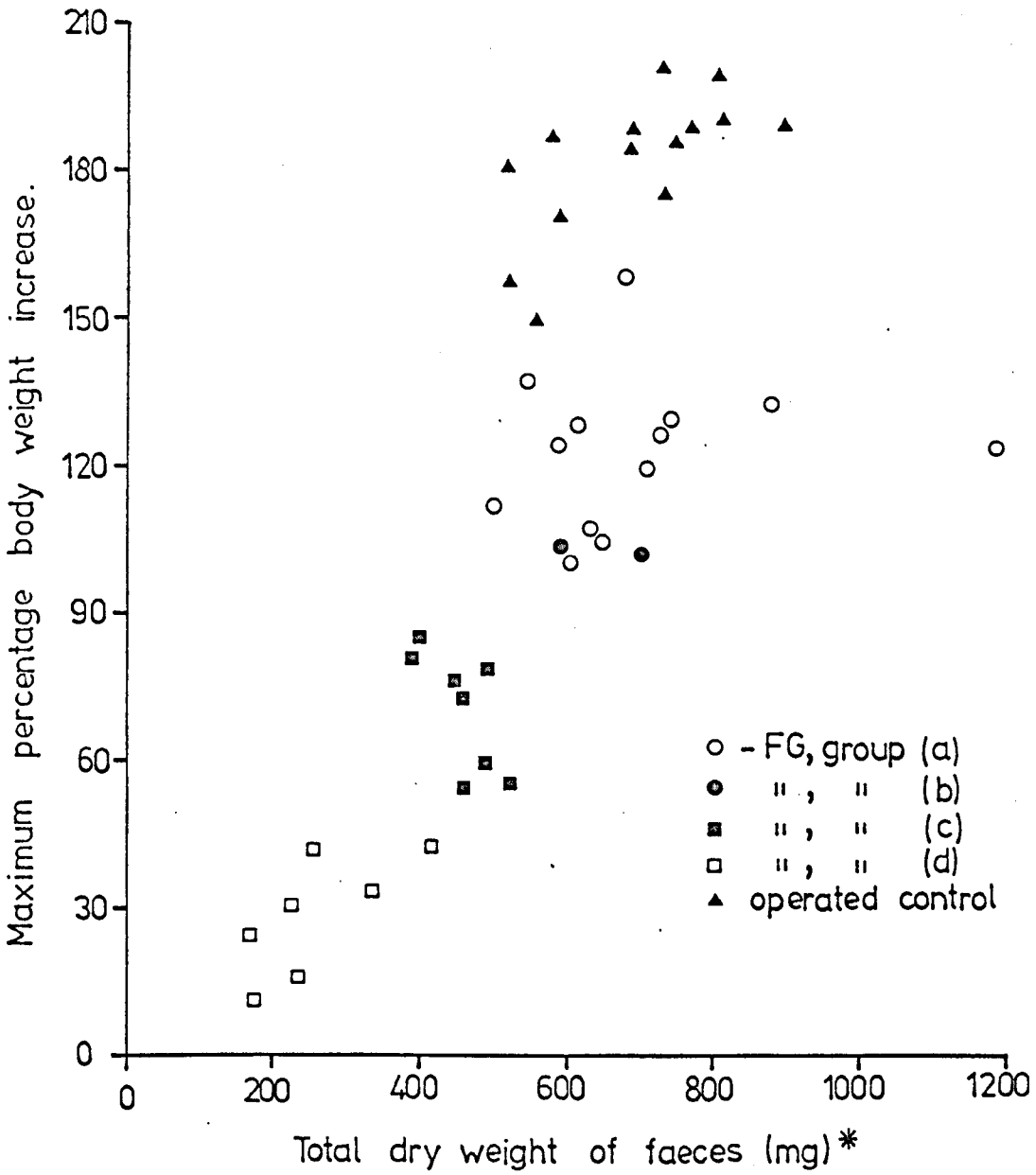
Three males and three females exhibited weight increases similar to those of group (b) animals but failed to develop a new cuticle, even after 26 days (Figs. 21 and 22). At autopsy the foregut of the majority of individuals was found to be packed full of undigested grass (Table 23). Daily faeces production and body weight increase in both sexes were much the same as in group (b) animals (Table 21).

(d) Little or no growth (0-50% increase in weight) and no attempted moulting

Two males and four females maintained an approximately constant weight after removal of the frontal ganglion (Figs. 21 and 22), and failed to develop a new cuticle. The foregut of every individual was found to be distended with undigested food at autopsy (Table 23). A constant low level of faeces production was manifest in animals of group (d) (Figs. 23 and 24) and this was associated with their low weight increases (Table 21). The peak(s) in faeces production witnessed in control and group (a) operated animals was absent in groups (c) and (d) operated animals.

The results in Section III show that frontal ganglionectomy has a variable effect on food passage, growth and cuticle development. The amount of growth taking place after the operation is directly related to the rate at which food leaves the foregut. True growth (minimum twofold increase in body weight) occurs when food passes through the gut unhindered; accumulation of food in the foregut results in little or no growth at all. Fig. 25 illustrates the relationship between the total amount of food passed through the gut (during the instar period in control and group (a) operated animals, and up until the time of death in groups (b), (c) and (d) operated animals) and maximum body weight gain in fifth instar animals of both sexes. It can be seen that group (a) operated animals tend to pass similar quantities of food through the gut to the controls but exhibit lower maximum body weight increases. This suggests that group (a) operated animals convert the ingested food into body tissue less efficiently than the controls. Part of the body weight gain in groups (c) and (d) operated animals of both instars is of course attributable to the accumulated, undigested food present in the foregut of these animals.

Frontal ganglionectomy also has a variable effect on the pattern of faeces production. Thus in group (a) operated animals, where food passes through the gut apparently unhindered, the peak in faeces production is readily distinguishable. On the other hand in groups (c) and (d) operated animals, where food passage out of the crop is restricted, the faeces peak is abolished altogether.



(* produced in the instar period in control and group (a) operated animals, and up until the time of death in groups (b), (c) and (d) operated animals).

FIG 25. The relationship between faeces production and body weight increase in frontal ganglionectomized and control fifth instar animals of both sexes.

The only long-term measurements of food consumption in frontal ganglionectomised locusts are those made by ROOME (1968) in third and fifth instar Locusta nymphs. Food consumption in operated animals of both instars was reduced to one-third to one-quarter that in the controls. The frontal ganglionectomised animals of ROOME maintained an approximately constant weight and displayed distended foreguts at autopsy; they therefore correspond to group (d) operated animals of the present work. From short-term feeding experiments BERNAYS and CHAPMAN (personal communication) found that frontal ganglionectomised fifth instar Locusta males consumed approximately half as much food as the controls during the course of a single meal.

CLARKE and LANGLEY (1963b) and CLARKE and GILLOTT (1965) state that frontal ganglionectomy in third instar Locusta nymphs does not adversely affect food intake and faeces production. Lack of growth after this operation is believed to be caused by a decrease in the activity of the neurosecretory system rather than be a lack of food. However, in the present study those operated animals failing to grow exhibit a correspondingly low level of faeces production, there being no examples of high faeces production and no growth. In contrast to the above authors HIGHNAM et al. (1966) report that removal of the frontal ganglion in immature adult female Schistocerca inhibits emptying of the foregut and consequently greatly reduces faeces production (HILL et al., 1966). Such operated animals are classed as 'semi-starved', and groups (c) and (d) animals of the present study fit into this category.

The frontal ganglionectomised fourth instar Locusta females of ROOME (1968) are divided into two groups according to their survival and growth responses: (1) 'short-lived' animals that survive for no longer than 10 days and exhibit a rapidly declining level of faeces production, which is associated with low weight increase and a distended crop at autopsy; (2) 'long-lived' animals that survive for longer than 10 days and show a relatively steady daily level of faeces production, which is more than double that of the short-lived animals but considerably less than that of the controls. By their growth responses, the long-lived animals of ROOME fit into groups (b), (c) and (d) (fourth instars) of the present work. Frontal ganglionectomy in Melanoplus (DOGRA and EWEN, 1971) has little or no effect upon food consumption, but severely restricts food passage out of the foregut. In consequence the crop becomes greatly distended and in a few individuals it actually bursts (GILLOTT et al., 1970).

The effects of frontal ganglionectomy on weight increase, new cuticle formation, and moulting behaviour are summarized in Table 24.

CLARKE and LANGLEY (1963c) removed the frontal ganglion from third instar nymphs at 0 hr, 12 hr, 24 hr, 33 hr, 72 hr and 96 hr after ecdysis. The 96 hr operated animals exhibited a steady weight loss until death. All the other operated animals maintained a constant weight and therefore correspond to group (d) operated animals of the present work. A new cuticle was developed by all of the 72 hr and 96 hr, and by a

TABLE 24 Weight increase, new cuticle formation and moulting behaviour in frontal ganglionectomised fourth and fifth instar Locusta nymphs

Instar	No. operated	No. surviving beyond Day 10	% animals surviving beyond Day 10 showing:				
			Weight increase (%)			New cuticle formation	Attempted moulting
			0-50	50-100	100		
5th	38	30	23	27	50	43	43
4th	30	17	35	53	12	29	29

few of the 33 hr operated animals. The 96 hr operated animals managed to split the old cuticle, but their attempts at moulting to the next stage proved unsuccessful. CLARKE and LANGLEY deduced that wound adhesions were responsible for this effect but a more likely explanation is failure of the operated animals first to empty the gut of food and second to swallow air.

ROOME (1968) removed the frontal ganglion from fourth instar Locusta females that were aged 8 hr ± 8 hr at the time of the operation. Of the nine animals surviving for longer than 10 days (long-lived), six exhibited weight increases similar to those of animals in groups (b) and (c) of the present work, while three showed weight increases which fit them into group (d). It is probable that had they not been sacrificed on Day 14 several of these long-lived animals would have increased their body weights sufficiently to fit into group (a). A new cuticle was developed by two of ROOME's long-lived animals.

Growth and new cuticle development can, therefore, still occur in the absence of the frontal ganglion, but successful moulting is blocked at all times.

SECTION IV

THE MORPHOLOGY OF THE ANTERIOR STOMATOGASTRIC NERVOUS SYSTEM

The earliest mention of the stomatogastric nervous system in Locusta is by BRANDT (1831) (as quoted in DUMORTIER, 1969). ALBRECHT (1953) provides the first detailed account of its structure and relationship with the brain and gut. His work has subsequently been extended by the studies of LANGLEY (1962), GRENVILLE (1962), CLARKE and LANGLEY (1963b, c), STRONG (1966) and ROOME (1968). The most comprehensive account of any orthopteran stomatogastric nervous system is that by WILLEY (1961) for Periplaneta. KHATTER (1968) describes at some length this system in another orthopteran, Schizodactylus monstrosus, while CAZAL (1948) frequently mentions the stomatogastric nervous system in his review of the arrangement of the retrocerebral glands in a wide variety of insect orders, including the Orthoptera.

The continued growth of some animals after removal of the frontal ganglion, as reported in Sections II and III, implies that sensory impulses produced by distension of the foregut must be reaching the brain and/or corpora cardiaca along

pathways other than those involving the frontal ganglion. An alternative route is suggested by STRONG (1966) and involves fine nerves leaving the surface of the pharynx ⟶ hypocerebral ganglion ⟶ nervi corporis cardiaci interni. Further routes are sought during the present investigation into the morphology of the anterior stomatogastric nervous system in Locusta. This part of the system includes the frontal connectives, frontal ganglion, recurrent nerve, hypocerebral ganglion and the various fine nerves connected to the frontal and hypocerebral ganglia. The structure and arrangement of the ingluvial ganglia, inner and outer oesophageal nerves and the fine nerves attached to ingluvial ganglia has been considered in Locusta by GRENVILLE (1962) and STRONG (1966), and in Schistocerca by DANDO et al. (1968).

Newly moulted fifth instar Locusta nymphs were starved for several days to remove fat tissue from the vicinity of the nerves. The locusts were then subjected to intra-vitam injection of reduced methylene blue according to the method of STARK et al. (1969). The following account is based on the results of some thirty dissections of injected specimens.

Muscles innervated by nerves of the anterior stomatogastric nervous system

ALBRECHT (1953) describes in detail the arrangement of the head muscles in Locusta and only a few additional observations are necessary here.

Situated just below the base of each antenna and adjacent to the frontal carina are two oval patches of cuticle which differ slightly in colour and pigmentation from the rest of the frons cuticle (Fig. 26). The lower patch, light brown in colour, is the external manifestation of the points of origin of four muscles: the anterior retractor of the labrum, the posterior retractor of the labrum, the retractor of the mouth angle and the second dorsal dilator of the pharynx (Figs. 27 and 28). The upper patch, yellow in colour, overlies the antennal ampulla (UVAROV, 1966). A broad, thin transverse band of muscle runs between the two antennal ampullae. Another band of muscle fans out ventrally from each ampulla and, passing outside the second dorsal dilator muscle of the pharynx and inside a large frontal air sac, inserts into the tunica muscularis close to the point of insertion of the first lateral dilator muscle of the pharynx (Fig. 29). In several orthopteran species (PAWLOWA, 1895 a, b) the walls of the aorta fuse with the transverse and vertical muscles of the ampullae. This also appears to be the case in Locusta.

FIG. 26 Relative positions of the upper and lower patches on the frons cuticle.

FIGS. 27 and 28. Dissections to show the muscles associated with the upper cuticular patch.

FIG. 29. Dissection to show the muscle bands associated with the antennal ampullae.

FIGS 26-29

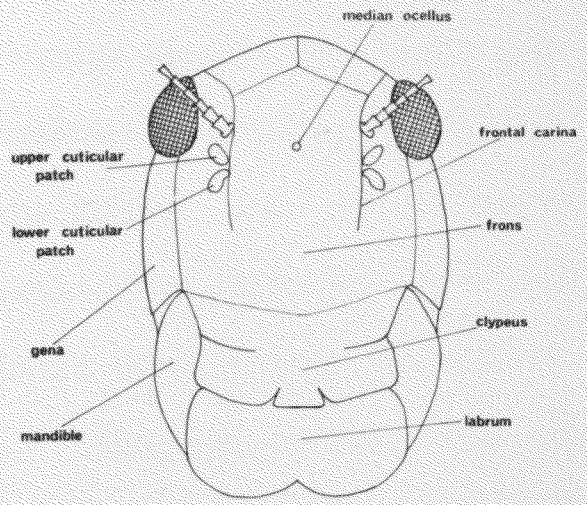


FIG 26

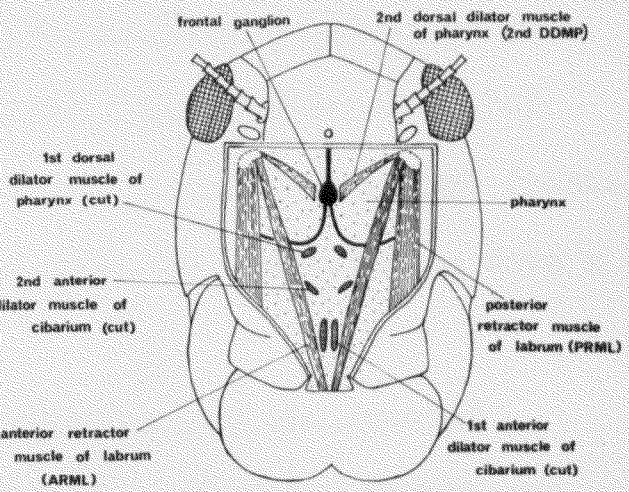


FIG 27

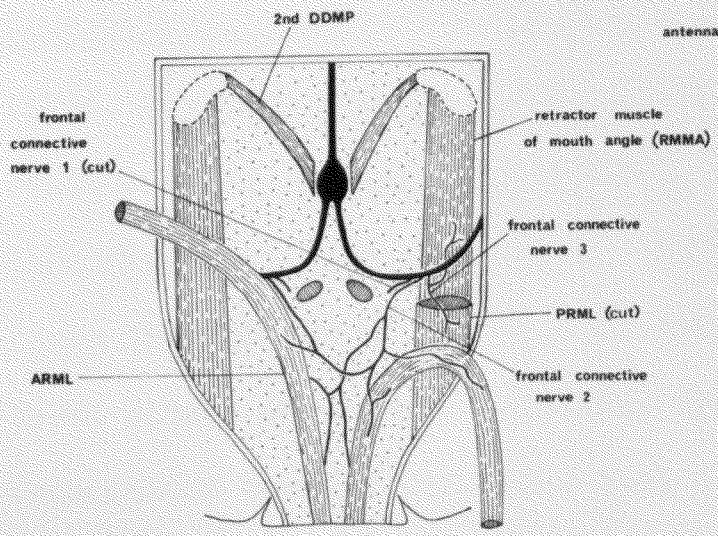


FIG 28

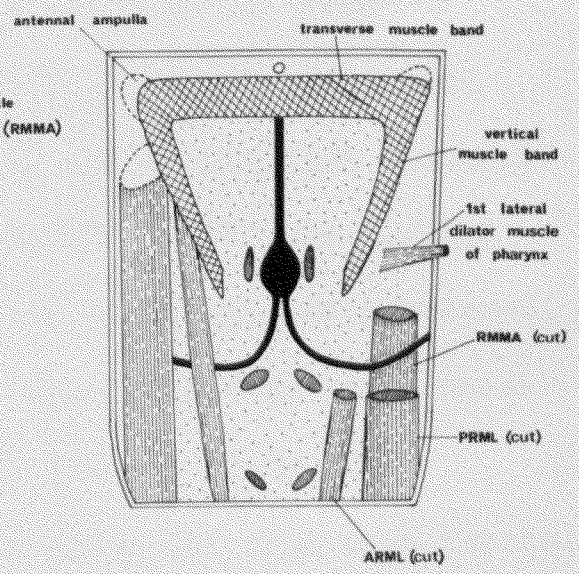


FIG 29

Frontal connectives and their branches

The frontal ganglion lies in the midline of the foregut between the second dorsal dilator muscles of the pharynx. Two frontal connectives leave its anterolateral margins and pass back on each side of the gut to the tritocerebral lobes of the brain. Just before entering the tritocerebrum each frontal connective fuses with the labral nerve on that side to form a short labrofrontal nerve root.

Fine nerves have been observed leaving the frontal connectives in Gryllotalpa gryllotalpa, Bacteria ferula and Blatta orientalis (BRANDT, 1835), Pachytylus migratorius, Stenobothrus bicolor and Forficula auricularia (PAWLOWA, 1895), Dixippus morosus (NYST, 1942), Naucoris cimicoides (CAZAL, 1948), Periplaneta (WILLEY, 1961), Blaberus craniifer (WILLEY, 1961), Schizodactylus (KHATTER, 1968) and Blabera fusca (BROUSSE-GAURY, 1971). As far as Locusta is concerned, ROOME (1968) observed fine nerves branching from the frontal connectives but did not trace them for any distance.

Usually three fine nerves are given off from each frontal connective in the region where this nerve loops back towards the tritocerebrum (Fig. 30, Plate 54). The first frontal connective nerve branch (FCN_1) runs forward to the second anterior dilator muscle of the cibarium and to the tunica

FIG. 30. Dorsal dissection of the nerve branches of the frontal connectives.

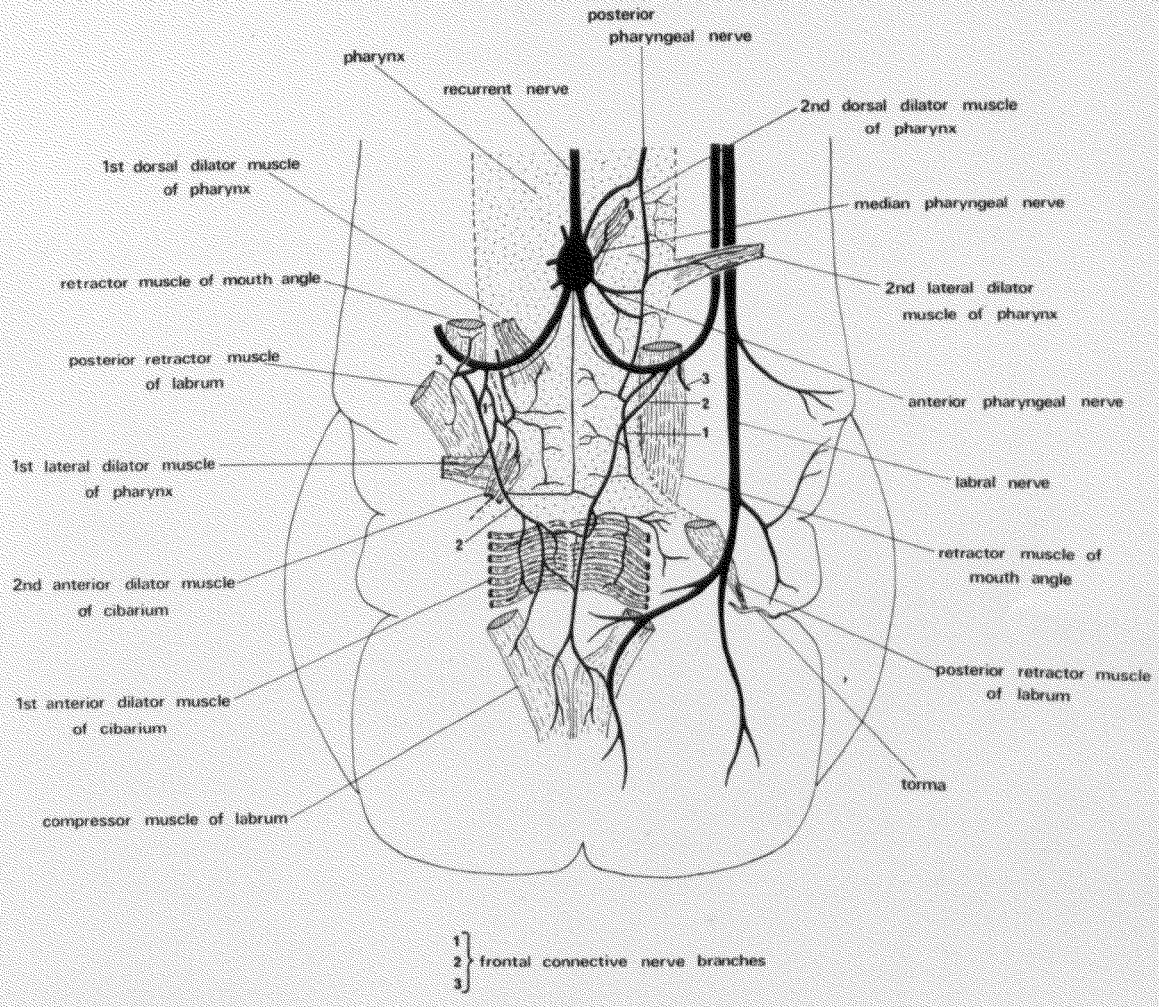

posterior pharyngeal nerve
pharynx
recurrent nerve
2nd dorsal dilator muscle of pharynx
1st dorsal dilator muscle of pharynx
median pharyngeal nerve
retractor muscle of mouth angle
2nd lateral dilator muscle of pharynx
posterior retractor muscle of labrum
anterior pharyngeal nerve
1st lateral dilator muscle of pharynx
labral nerve
retractor muscle of mouth angle
2nd anterior dilator muscle of cibarium
posterior retractor muscle of labrum
1st anterior dilator muscle of cibarium
torma
compressor muscle of labrum
1
2
3
frontal connective nerve branches

FIG 30

PLATE 54. Methylene blue injection showing branches of the frontal connectives.

Frontal connective nerve 1 (FCN_1); Frontal connective nerve 2 (FCN_2); Frontal connective nerve 3 (FCN_3). A median nerve (MN) runs from the frontal ganglion to the FCN_1. Note also the fine nerve (FN) linking the FCN_2 to the labral nerve, and a distal branch of the frontal connective (DBFC) joining up with the FCN_2.

Whole mount. x 25.

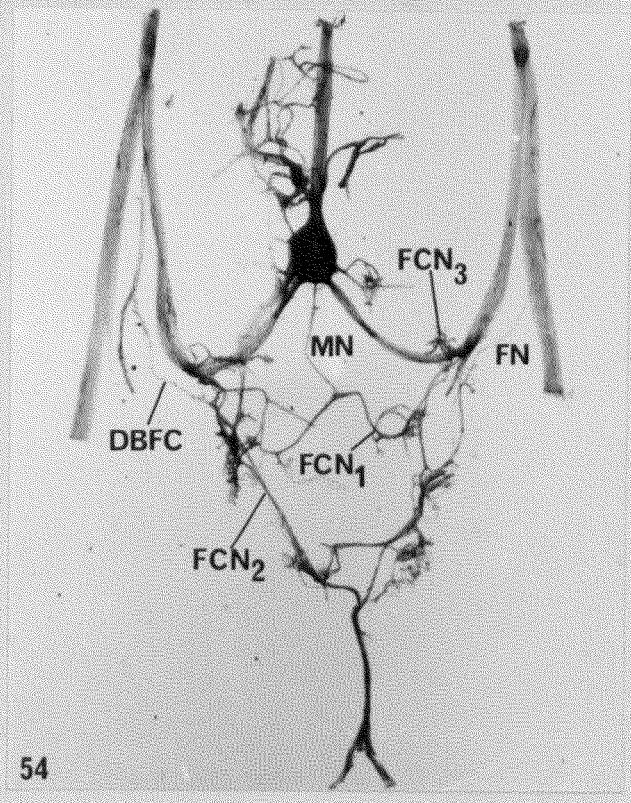
PLATE 55. Methylene blue injection showing distribution of the posterior pharyngeal nerve (PPN).

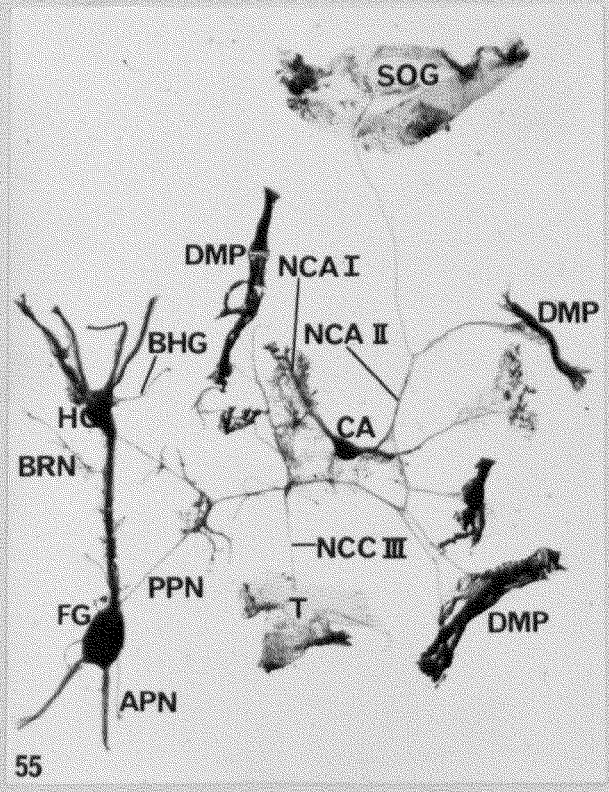
This nerve branches to the hypocerebral ganglion (HG), tritocerebrum (T), nervus corporis allatum II (NCA II), surface of the pharynx, and dilator muscles of the pharynx (DMP).

Note also the frontal ganglion (FG), anterior pharyngeal nerve (APN), branch of the recurrent nerve (BRN), branch of the hypocerebral ganglion (BHG), corpus allatum (CA), nervus corporis allatum I (NCA I), nervus corporis cardiacum III (NCC III), and sub-oesophageal ganglion (SOG).

Whole mount. x 20.

PLATES 54, 55


FCN3
MN
FN
DBFC
FCN1
FCN2
54


SOG
DMP
NCA I
DMP
BHG
NCA II
HG
CA
BRN
NCC III
PPN
T
DMP
FG
APN
55

muscularis of the clypeal epipharynx and the pharynx proper. Sensory cell bodies, whose axons connect with the FCN_1, can be detected lying on the tunica muscularis and occasionally on the dilator muscles. Motor nerve nedings of the FCN_1 branches are also evident in these muscles (Plate 56). A branch of the FCN_1 passes back under the frontal connective, branches to the first dorsal dilator muscle of the pharynx and then links up with the anterior pharyngeal nerve (Fig. 30).

The second frontal connective nerve branch (FCN_2) passes anteriorly and branches extensively to supply the following muscles: posterior retractor of the labrum, retractor of the mouth angle, anterior retractor of the labrum, first and second anterior dilators of the cibarium, compressor of the labrum and the tunica muscularis of the clypeal epipharynx (Fig. 30, Plate 54). Sensory cell bodies, whose axons connect with the FCN_2, are located on the tunica muscularis and occasionally on the dilator muscles while motor endings of the FCN_2 branches are common in both muscle regions. The main FCN_2 nerve branch may unite with its opposite number before innervating the compressor of the labrum or the two may be joined by 'link' nerves. The FCN_2 is probably homologous to the 'N_2' of WILLEY (1961).

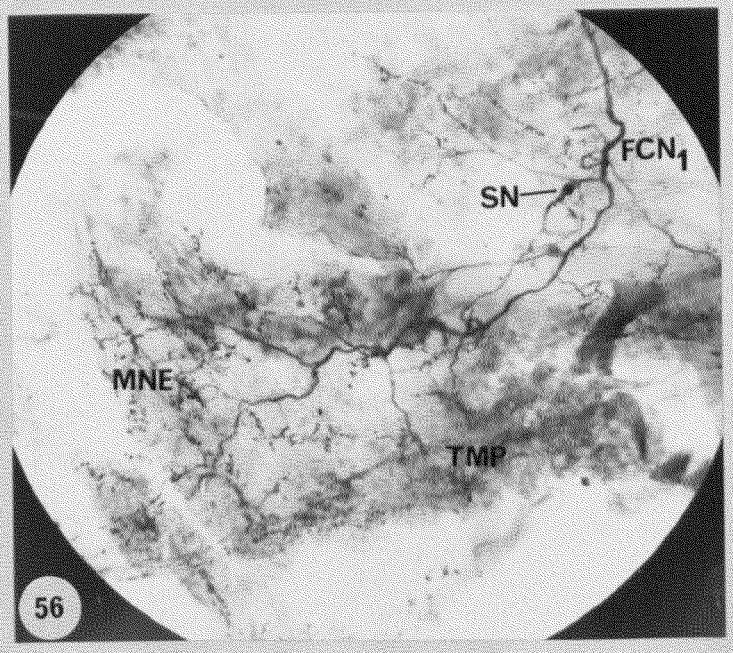
PLATE 56. Sensory neurone (SN) associated with a branch of the frontal connective nerve 1 (FCN_1).
Motor nerve endings (MNE); Tunica muscularis of the pharynx (TMP).

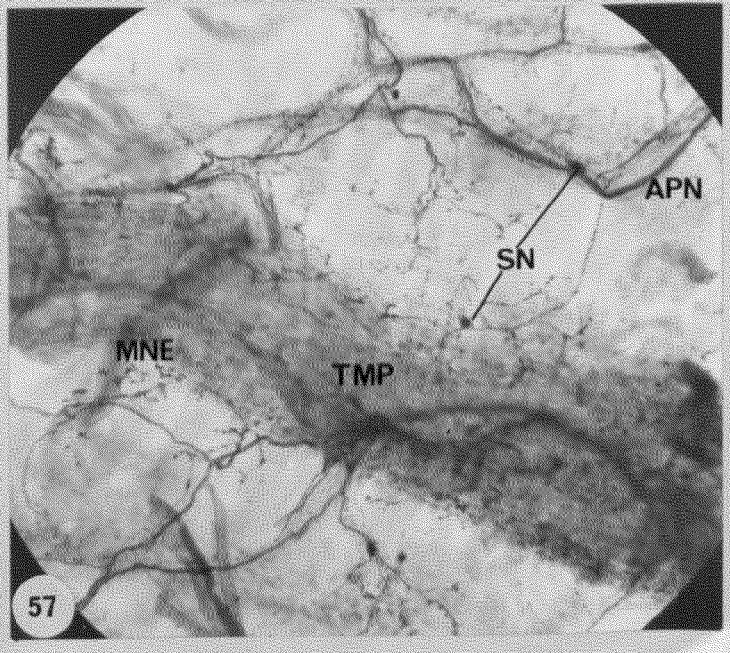
Whole mount. Methylene blue injection. x 135.

PLATE 57. Sensory neurones (SN) associated with branches of the anterior pharyngeal nerve (APN).
Motor nerve endings (MNE); Tunica muscularis of the pharynx (TMP).

Whole mount. Methylene blue injection. x 135.

PLATES 56, 57





The third frontal connective nerve branch (FCN_3) divides to form an anterior branch which innervates the posterior retractor of the labrum and a posterior branch which innervates the rectractor of the mouth angle. This nerve is probably homologous to the 'N_1' of WILLEY.

While the FCN_3 and FCN_2 nearly always arise as separate branches of the frontal connective, the FCN_1 may branch from the FCN_2 instead of from the frontal connective.

Several other fine nerves were observed leaving the frontal connectives but could not be regularly identified in every dissected specimen. Thus, on one side only, a nerve occasionally leaves the frontal connective just before it fuses with the labral nerve (Fig. 30, Plate 54). The nerve branch divides into two, one half connecting with the FCN_2 and the other extending to the inner epithelium of the clypeus. WILLEY (1961) for Blaberus and KHATTER (1968) for Schizodactylus figure a nerve leaving the frontal connective just before it fuses with the labral nerve. In Schizodactylus the nerve branch supplies the pharyngeal muscles.

One or both frontal connectives occasionally branch just before they enter the frontal ganglion. The branch may re-enter the frontal connective or join the FCN_1 (Fig. 30).

The frontal connective and labral nerve are often connected by very fine 'link' nerves (Fig. 30).

Anterior, median and posterior pharyngeal nerves

The anterior and posterior pharyngeal nerves were first described in Locusta by CLARKE and LANGLEY (1963 b, c). A third pair of fine nerves, the median pharyngeal nerves, are given off from the frontal ganglion between the anterior and posterior pairs (ROOME, 1968). Fine nerves leaving the frontal ganglion between the frontal connectives and the recurrent nerve are also described for Polyzosteria nitida, Gryllus domesticus, Stenobothrus and Forficula (PAWLOWA, 1895), Hierodula bioculata and Gryllotalpa vulgaris (BORDAS, 1900), Oryctes nasicornis (PAWLOWA, 1895; ORLOV, 1924), Carausius morosus (DUPONT-RAABE, 1957), Periplaneta (WILLEY, 1961; DAVEY and TREHERNE, 1963), Schizodactylus (KHATTER, 1968) and Blabera (BROUSSE-GAURY, 1971).

The anterior pharyngeal nerves (APNs) emerge from the anterolateral borders of the frontal ganglion, just behind the origin of the frontal connectives. They innervate the first dorsal and lateral dilator muscles of the pharynx, the rectractor muscles of the mouth angle and the tunica muscularis of the pharynx (Fig. 30). Axons of sensory cell bodies found lying on the tunica muscularis and occasionally on the dilator muscles connect with the APNs. Motor nerve endings of the fine branches of the APNs are also apparent in these muscles (Plate 57).

The median pharyngeal nerves (MPNs) either emerge separately from the frontal ganglion, just posterior to the APNs, or arise as branches of the APNs. They innervate the second dorsal dilator muscles of the pharynx (Fig. 30).

The site of origin of the posterior pharyngeal nerves (PPNs) is, in comparison to that of the APNs, unpredictable. Thus, both PPNs may arise from the frontal ganglion, both from the recurrent nerve or one from the frontal ganglion and one from the recurrent nerve. The PPNs branch profusely over the surface of the pharynx and oesophagus to innervate the second and third dorsal dilator muscles of the pharynx, the second lateral dilator muscles of the pharynx, the first, second and third ventral dilator muscles of the pharynx, the thin vertical muscle sheets associated with the antennal ampullae and the tunica muscularis of the pharynx (posterior region) and oesophagus. The PPNs also connect with branches of the APNs and MPNs, branches of the recurrent nerve, branches of the hypocerebral ganglion, the nervi corporis cardiaci III (NCC III), the corpora cardiaca, the nervi corporis allati I and II (NCA I and II) and also the aorta (Fig. 31, Plate 55). Sensory cell bodies whose axons connect with the PPNs can be detected lying on the surface of the tunica muscularis and occasionally on the dilator muscles. Motor nerve endings of the fine branches of the PPNs are also apparent in these muscles (Plate 58).

FIG. 31. Dorsal dissection of the posterior pharyngeal nerve.

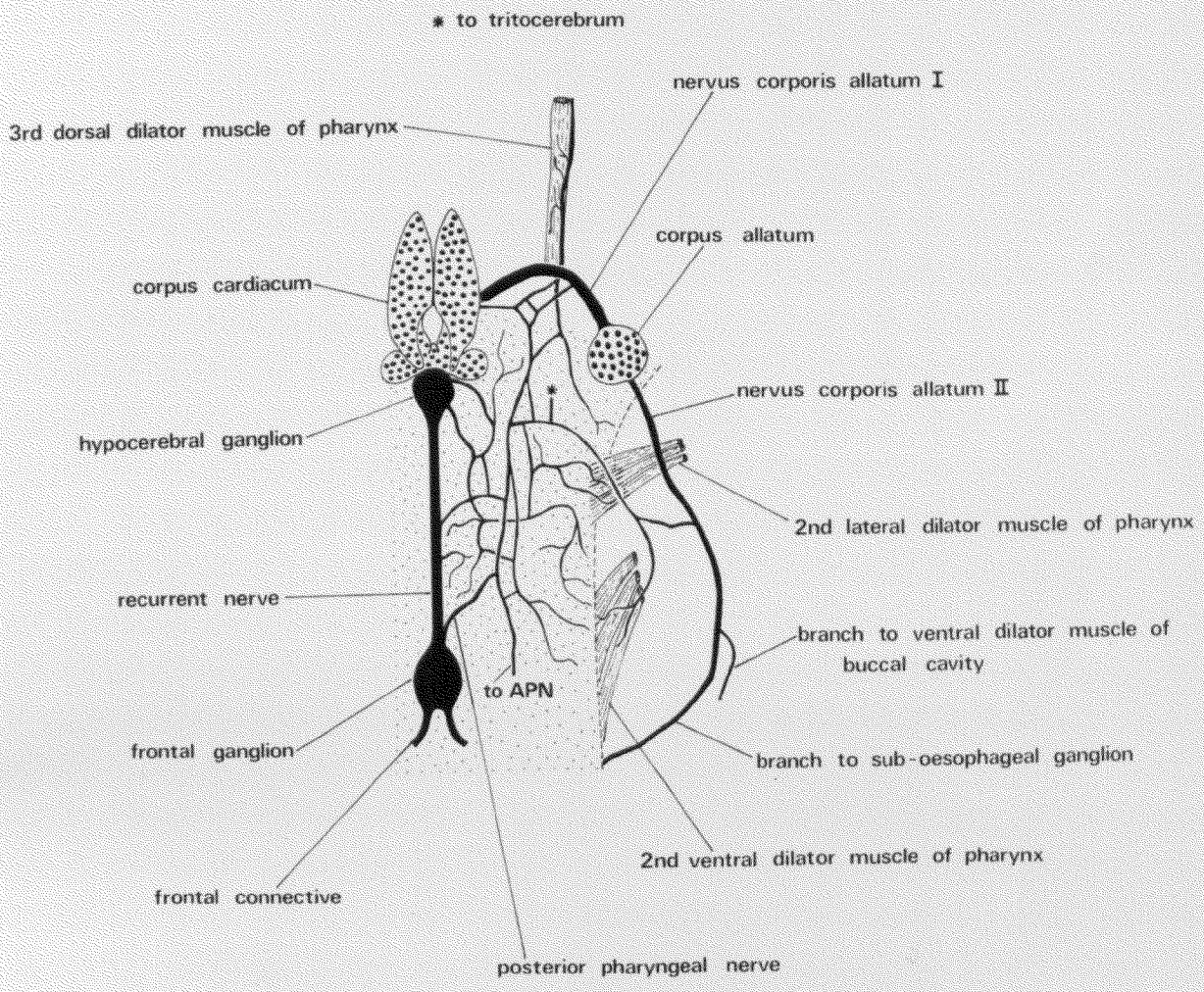

* to tritocerebrum
nervus corporis allatum I
3rd dorsal dilator muscle of pharynx
corpus allatum
corpus cardiacum
nervus corporis allatum II
hypocerebral ganglion
2nd lateral dilator muscle of pharynx
recurrent nerve
branch to ventral dilator muscle of buccal cavity
to APN
frontal ganglion
branch to sub-oesophageal ganglion
2nd ventral dilator muscle of pharynx
frontal connective
posterior pharyngeal nerve

Fig. 21

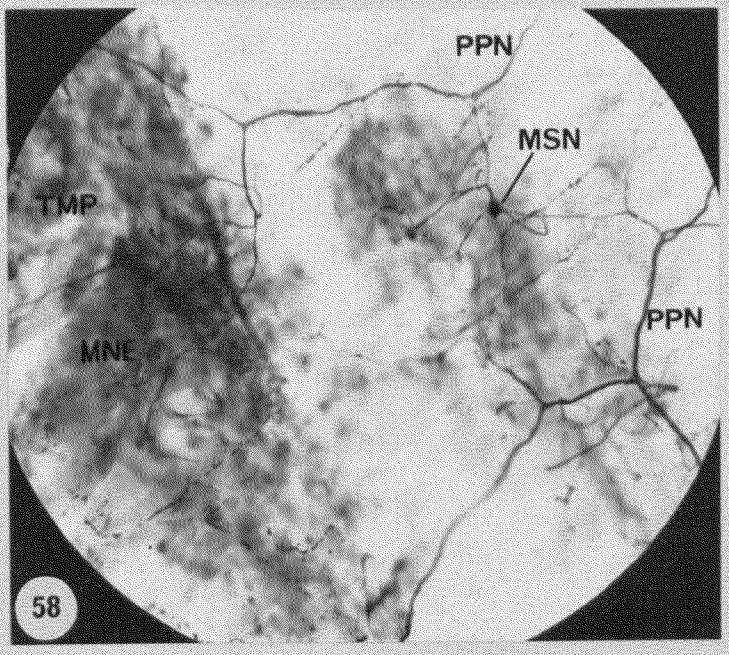
PLATE 58. Multiterminal sensory neurone (MSN) associated with branches of the posterior pharyngeal nerve (PPN). Motor nerve endings (MNE); Tunica muscularis of the pharynx (TMP).

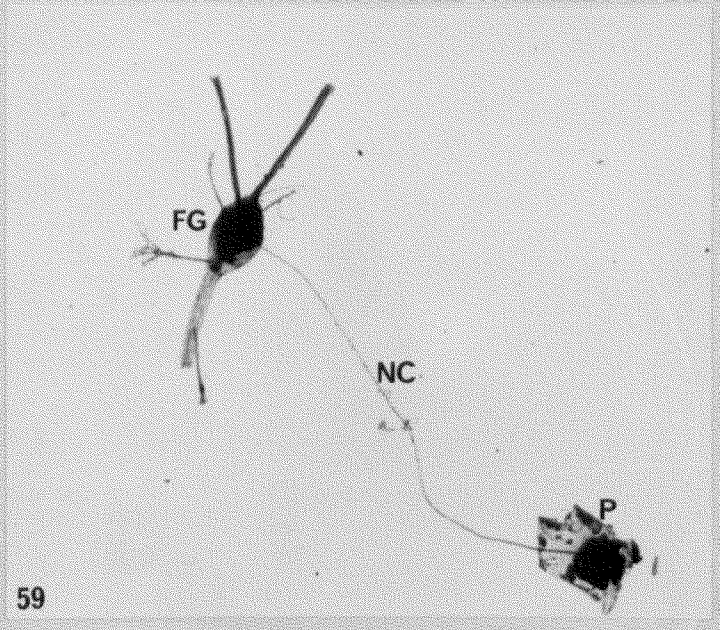
Whole mount. Methylene blue injection. x 135.

PLATE 59. Methylene blue injection showing the nervus connectivus (NC). This nerve runs from the protocerebrum (P) to the frontal ganglion (FG).

Whole mount. x 25.

PLATES 58, 59





LANGLEY (1962) and CLARKE and LANGLEY (1963b) describe a nerve leaving the NCA II and branching to the ventral dilator muscles of the pharynx, the muscles of the ventral head apodeme and the tritocerebrum. RAABE (1963b, 1964), also for Locusta, depicts a nerve branching from the NCA II to the ventral dilator muscles of the pharynx and shows the NCC III branching to the corpora allata and corpora cardiaca. She adds that in Locusta, Aeschna grandis, Carausius and Gryllus the NCC III branches to the dilator muscles of the pharynx and in the latter three insects it also connects with the recurrent nerve. From the present study it is clear that the nerve CLARKE and LANGLEY observed branching to the tritocerebrum is almost certainly the NCC III and the branch of the NCC III said by RAABE to innervate the dilator muscles of the pharynx is in fact part of the PPN. In Carausius (DUPONT-RAABE, 1957) the PPN supplies the dilator muscles of the pharynx and also joins the NCC III, the combined nerve root then entering the corpora cardiaca. The equivalent of the PPN in Periplaneta, 'N_5' of WILLEY (1961), is reported by this author only to innervate the tunica muscularis of the pharynx, there being no obvious link up with the NCC III. According to DAVEY and TREHERNE (1963) the 'N ' in this insect terminates in a sensory organ which they believe to be an osmoreceptor. These authors also record a single median nerve passing posteriorly from

the ventral surface of the frontal ganglion to the muscular coat of the pharynx; no such nerve exists in Locusta.

Nervus connectivus

A slender median nerve, the nervus connectivus, arises from the mid-dorsal surface of the frontal ganglion. It passes back through the transverse muscle band connecting the antennal ampullae and enters the protocerebrum (Plate 59). The nervus connectivus was first named by BALDUS (1924) in the dragonfly Aeschna, although PAWLOWA (1895) had previously figured the nerve in various Orthoptera, labelling it 'n_3'. CAZAL (1948) reviews the occurrence of this nerve throughout the Insecta and finds it to be a characteristic feature of the more primitive orders. According to WILLEY (1961) the nervus connectivus is found in all orthopteroids except the Saltatoria but this nerve has not previously been described in Locusta migratoria migratorioides. In Periplaneta (WILLEY, 1961) the nervus connectivus is composed of two large axons which belong to a pair of large unipolar cell bodies situated on the median anterior face of the protocerebrum. The nervus connectivus does not branch and its function remains undetermined.

Other fine nerves leaving the frontal ganglion

In many insects a single median nerve, the frontal nerve (IMMS, 1957), emerges from the anterior margin of the frontal ganglion and runs forward over the dorsal surface of the pharynx and cibarium innervating various muscles in these regions. CLARKE and LANGLEY (1963c) provide a list of insects in which the frontal nerve has been detected. To it may be added the following species: Hyloicus ligustri (NEWPORT, 1832), Bombyx mori (BLANC, 1890), Forficula (PAWLOWA, 1895a), eight different orthopteran species (BORDAS, 1900), Grylloblatta campodiciformes (NESBITT, 1956), Dendroctonus pseudotsugae (ATKINS and CHAPMAN, 1957), Dytiscus marginalis (RAABE, 1963 a), Schizodactylus (KHATTER, 1968) and Actias selene (ROOME, 1968). A single median nerve is also observed in Locusta, though not in every dissected specimen. When present it seems to connect with the FCN_1 (Fig. 30, Plate 54) or the frontal connective. An alternative arrangement is apparent in Plates 60a, b where a multiterminal neurone is seen to be closely associated with the FCN_2. The dedritic processes of the neurone run on and in this nerve while the axon enters the frontal ganglion midway between the points of origin of the frontal connectives. Multiterminal neurones have also been identified on major nerves in Carausius (FINLAYSON and OSBORNE, 1968). The

PLATE 60a. A multiterminal neurone (MTN) associated with the frontal connective nerve 2 (FCN_2). The axon of this nerve cell runs to the frontal ganglion (FG).

Whole mount. Methylene blue injection. x 25.

PLATE 60b. The same at higher power. Note the dendritic processes (DP) intermingling with the FCN_2.

Whole mount. Methylene blue injection. x 400.

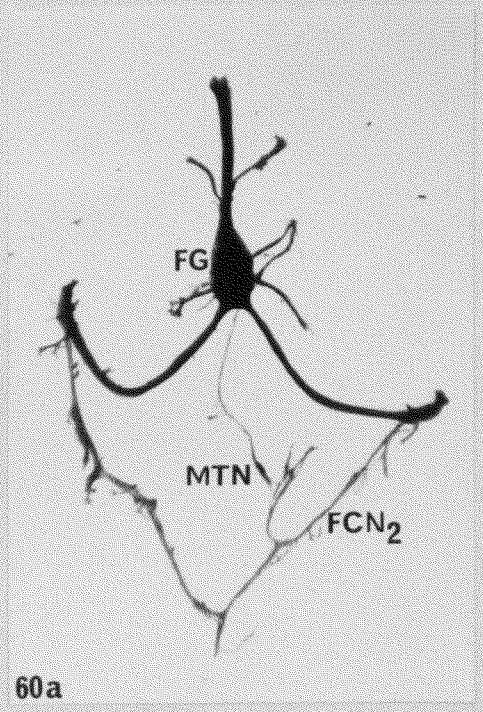
PLATE 61a. Showing large number of nerves associated with the frontal ganglion.

Whole mount. Methylene blue injection. x 40.

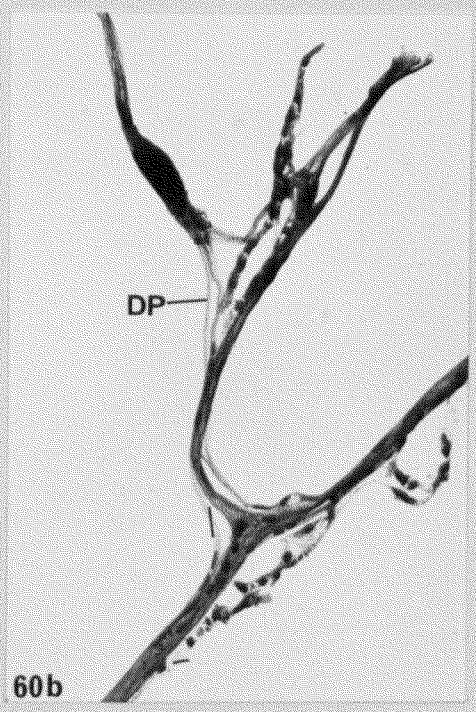
PLATE 61b. Showing nervous continuity between the anterior pharyngeal nerve (APN), posterior pharyngeal nerve (PPN) and a branch of the recurrent nerve (BRN). Note also the median pharyngeal nerve (MPN) and sensory neurone (SN).

Whole mount. Methylene blue injection. x 60.

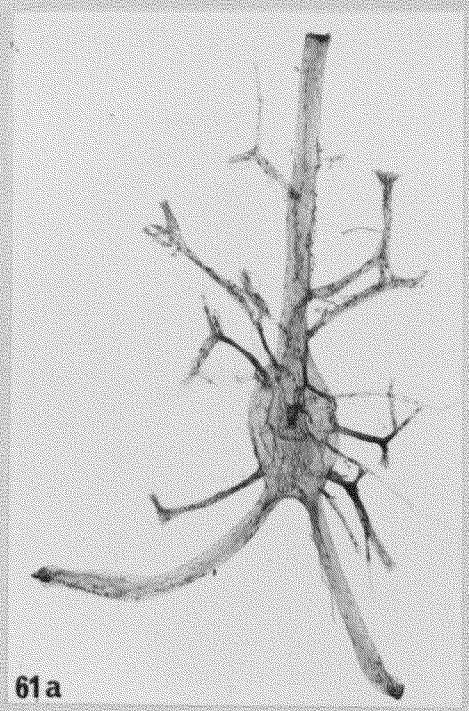
PLATES 60 - 61



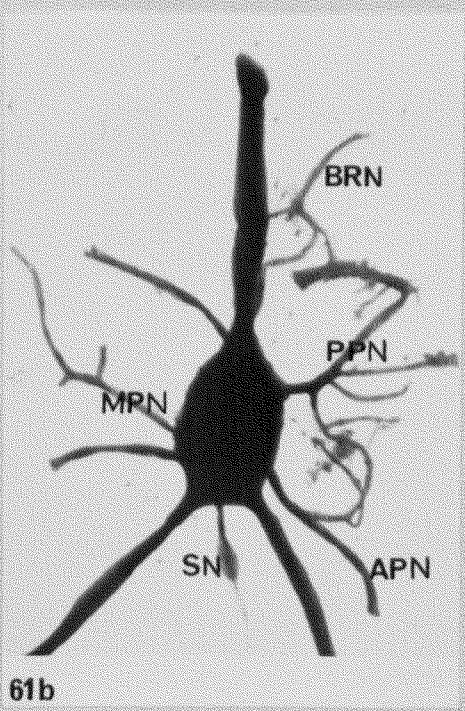
60a



60b



61a



61b

muscles innervated by the frontal nerve in, for example, Schizodactylus are, in Locusta, supplied by the FCN_1 and FCN_2.

Two fine nerves leave the frontal ganglion between the frontal connectives and run forward over the surface of the gut in the Odonata (BRANDT, 1838), Pachytylus, Polyzosteria and Oryctes (PAWLOWA, 1895), Dixippus (NYST, 1942) and Forficula and Sialis lutaria (CAZAL, 1948). In Gryllus (PAWLOWA, 1895) and Gryllotalpa (BORDAS, 1900) three fine nerves arise just in front of the points of origin of the frontal connectives. In Periplaneta (WILLEY, 1961) the number is four ('N_3' and 'N_4') while in Schizodactylus (KHATTER, 1968) it is six (not including the frontal nerve).

Plate 61a shows a frontal ganglion with all its nerve connections intact. The single anteriorly directed median nerve is absent and the nervus connectivus has been cut near its base. The extremely fine nerves are probably axons of sensory cell bodies occasionally found lying on fat body tissue and against the frontal air sacs. FINLAYSON and OSBORNE (1968) describe a fat body neurone in Carausius whose axon enters a nerve connected to the second abdominal ganglion; its function is unknown.

Recurrent nerve and its branches

The frontal ganglion narrows posteriorly into the recurrent nerve trunk which passes back along the mid-dorsal line of the gut to the hypocerebral ganglion. Several (the number is variable) fine nerves leave the recurrent nerve and link up with the branches of the PPNs (Plate 61b). Some of these branches then innervate the tunica muscularis of the pharynx and oesophagus (Fig. 31). In Stenobothrus (PAWLOWA, 1895a), Oryctes (ORLOV, 1924), Periplaneta (WILLEY, 1961), Schizodactylus (KHATTER, 1968) and Actias (ROOME, 1968) branches of the recurrent nerve innervate the muscle coat of the pharynx and oesophagus. In Dytiscus (RAABE, 1963 b) a pair of fine nerves emerge from the recurrent nerve and supply the lateral dilator muscles of the pharynx while in Gryllus, Aeschna and Carausius (RAABE, 1963 b) branches of the recurrent nerve connect with the NCC III. A fine nerve branch, containing two bipolar neurones, arises from the ventral surface of the recurrent nerve in Phormia (GELPERIN, 1967) and connects with the anterior region of the foregut. From electrophysiological experiments it is concluded that the two neurones are stretch receptors.

Nerves connected to the hypocerebral ganglion

The hypocerebral ganglion lies on the dorsal surface of the oesophagus, just behind the brain and immediately underneath the anterior unpaired lobe of the corpora cardiaca. Its neuropile is continuous with the recurrent nerve, the inner and outer oesophageal nerves, branches of the NCC I and small nerves to the pharynx (STRONG, 1966). The small or fine nerves usually link up with branches of the PPNs before innervating the muscle coat of the oesophagus (Fig. 31, Plate 55). Fine nerves leaving the hypocerebral ganglion and passing to the surface of the oesophagus are also described for Dixippus (PFLUGFELDER, 1937; NYST, 1942), Periplaneta (WILLEY, 1961), Schizodactylus (KHATTER, 1968), and Oryctes, where they connect with branches of the recurrent nerve and PPNs (ORLOV, 1924). In Grylloblatta (NESBITT, 1956) fibres from the hypocerebral ganglion innervate the aorta.

The above neuromorphological study has shown that the necessary sensory and motor nerve elements are present in the pharynx and oesophagus for the co-ordinated control of muscular activity in these regions. On the basis of work carried out in other insects by ZAWARZIN (1916) and DANDO et al. (1968), the multipolar or multiterminal (FINLAYSON,

1968) neurones found lying on the surface of the pharynx and oesophagus in Locusta can be classed as Type II sensory cells. CLARKE and LANGLEY (1963c) and PLOTNIKOVA (1967) go further and suggest that these cells function as stretch receptors, although this still awaits electrophysiological confirmation.

In the light of the present investigation the account by LANGLEY (1962) and CLARKE and LANGLEY (1963c) on the distribution of the PPNs is now seen to be inaccurate. The region of the pharynx described by these authors as being innervated by the PPNs is, in fact, supplied by the APNs and FCN_1, with the PPNs supplying that region of the foregut which lies posterior to the frontal ganglion. The PPNs branch extensively and link the stomatogastric nervous system with the neuroendocrine system at the level of the corpora cardiaca and also at the level of the NCA II.

The distribution of the fine nerves of the anterior stomatogastric nervous system is such that even after the removal of the frontal ganglion several nervous pathways are open to the insect for the conveyance of sensory information from the foregut to the neuroendocrine system. The FCN_1, FCN_2, APN and PPN are mixed nerves. In Section V the ultrastructure of one of these, the PPN, is investigated and its axon diameters measured.

SECTION V

AN ELECTRON MICROSCOPICAL EXAMINATION OF THE HYPOCEREBRAL GANGLION, INGLUVIAL GANGLION, FRONTAL CONNECTIVE, RECURRENT NERVE AND POSTERIOR PHARYNGEAL NERVE

Neurosecretory material cannot be detected in the intrinsic neurones of the stomatogastric nervous system in Locusta at the light level (GRENVILLE, 1962; LANGLEY, 1962; CLARKE and LANGLEY, 1963 c; STRONG, 1966). STRONG did, however, observe several neurosecretory axons from the nervi corporis cardiaci interni enter the neuropile of the hypocerebral ganglion and leave in the inner and outer oesophageal nerves. These nerves in Melanoplus sanguipipes also contain neurosecretory fibres from the nervi corporis cardiaci interni (DOGRA and EWEN, 1970). The only successful light microscopical demonstration of intrinsic neuroglandular cells in the stomatogastric nervous system in any insect is by VAN DER KLOOT (1960) for the frontal ganglion of Bombyx mori.

A quite different picture is manifest at the ultrastructural level. ANSTEE (1968) found the perikarya of the frontal ganglion in Locusta to be actively engaged in the elaboration of neurosecretory granules which he also detected

in many of the axons within the neuropile of this ganglion. His observations were confirmed by CAZAL et al. (1971) who in addition discovered neurosecretory material in the hypocerebral ganglion of this insect. CHANUSSOT et al. (1969) provide pictorial evidence of the existence of neurosecretion in the ingluvial ganglion of Blabera craniifer and describe its presence in this ganglion in Schistocerca.

ANSTEE (1968) inspected a limited number of thin sections of the recurrent nerve but was unable to detect neurosecretory material in any of its axons. The recurrent nerve runs between the frontal ganglion and the hypocerebral ganglion and since both of these ganglia actively manufacture neurosecretion its non-appearance in the recurrent nerve is somewhat surprising. The recurrent nerve therefore merits further attention.

In this section the fine structure of the frontal ganglion, as described by ANSTEE (1968) and CAZAL et al. (1971), is compared with that of the hypocerebral and ingluvial ganglia, the account by CAZAL et al. of ultrastructure of the hypocerebral ganglion being of sufficient brevity to justify this further investigation. The frontal connective, recurrent nerve and posterior pharyngeal nerve, all of which were cut during various experiments described in Sections I and II, are also examined at the ultrastructural level. The

required tissues in mid-fourth instar nymphs and 10-day-old adults were fixed and processed for electron microscopy according to the methods described in Chapter II. For each tissue sections were taken of six animals and the results presented below are representative, in each case, of over one hundred cell profiles. Plates 75, 77, 81, 85 and 87 are of fourth instar animals while the remaining plates in this section are of adults. Since nerve axons and neurosecretory granules are rarely perfectly circular in transverse section the figure given for 'diameter' is the mean of the two diameters (WIGGLESWORTH, 1959).

Hypocerebral Ganglion

The hypocerebral ganglion is invested with a nerve sheath comprising an outer acellular layer, the neural lamella, and an inner cellular layer, the perineurium. Concentrated mainly round the dorsal and dorso-lateral aspects of the medullary neuropile are the cortical neurone cell bodies or perikarya. The sub-perineurial glial system ensheaths the neuronal perikarya and extends as narrow processes into the neuropile.

The nerve sheath

The structure of the neural lamella resembles that of its counterpart in the metathoracic ganglion (ASHHURST and CHAPMAN, 1961). It therefore consists of a meshwork of collagen-like fibrils set in an amorphous background substance which is frequently penetrated by tracheoles. According to ASHHURST (1959) the background substance is a neutral mucopolysaccharide.

The fine structure of insect perineurial cells has been described by HESS (1958), ASHHURST and CHAPMAN (1961), TRUJILLO-CENOZ (1962), SMITH and TREHERNE (1963), MADDRELL and TREHERNE (1967) and SMITH (1967). In Locusta the distinctive features of the perineurial cells are the abundant rod-shaped mitochondria and the large intercellular spaces which are produced when adjacent pairs of intercellular membranes diverge widely from one another (Plate 62). The intercellular clefts, which open directly under the neural lamella, are held together at their inner ends by septate desmosomes. Also present in the cytoplasm are scattered Golgi complexes, profiles of rough endoplasmic reticulum, free ribosomes, sparsely distributed clusters of glycogen granules and microtubules. The perineurium nucleus is sausage-shaped and contains dense clumps of chromatin material.

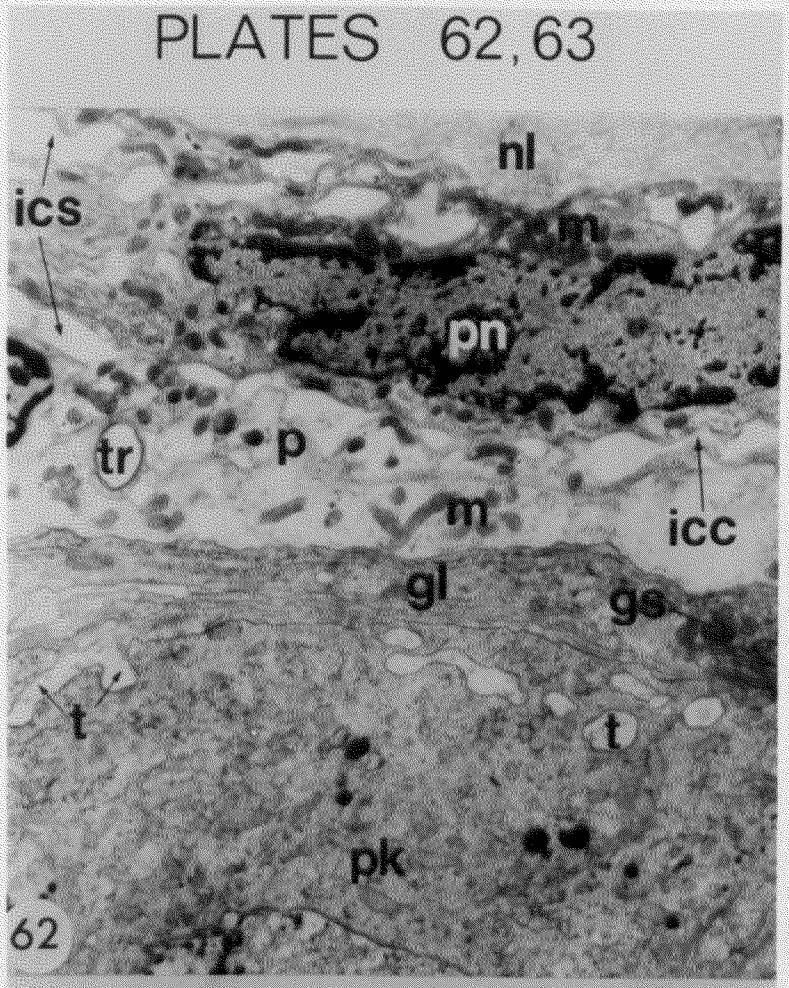
PLATE 62. Electron micrograph of the cortical region of the hypocerebral ganglion. At the top of the field lies the neural lamella (nl) and below this the perineurium (p). The characteristic intercellular clefts (icc) between adjacent perineurial cells periodically diverge to form large intercellular spaces (ics). Multiple closely packed sheets of glia (gl) separate the perineurium from the perikaryon (pk). The innermost of these sheets invaginates the perikaryal nucleus (pn), mitochondria (m), tracheole (tr) and gliosome (gs).

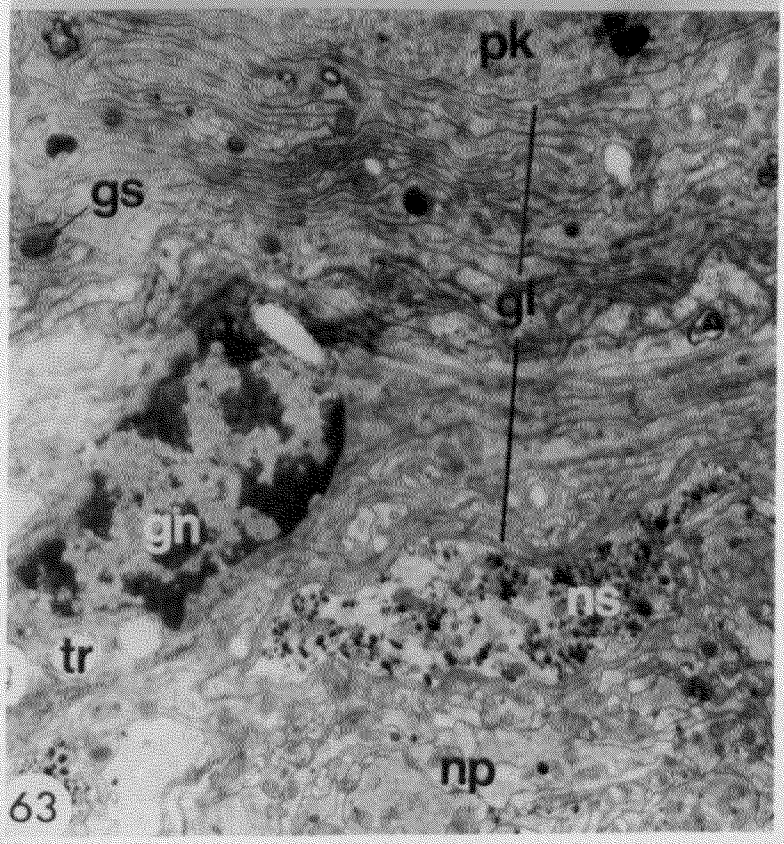
Magnification x 9,400.

PLATE 63. A section taken through the edge of the hypocerebral ganglion neuropile. Numerous stacked leaflets of glial cells (gl) separate the perikaryon (pk) from the neuropile (np). The glial cytoplasm contains a glial nucleus (gn), tracheoles (tr) and a gliosome (gs). Note the large neurosecretory axon (ns) in the neuropile.

Magnification x 9,400.

PLATES 62, 63





The neuroglia

The structure and function of the glial cell system in insects has been reviewed by SMITH and TREHERNE (1963), SMITH (1967), TREHERNE (1967) and TREHERNE and MORETON (1970). In the hypocerebral ganglion the attenuated cytoplasmic processes of the glial cells form concentric folds round the neurone cell bodies and the innermost of these glial sheets penetrates the peripheral cytoplasm of the perikaryon to form characteristic invaginated 'glial fingers' (Plate 62). Within the glial cytoplasm may be found profiles of rough endoplasmic reticulum, free ribosomes, scattered Golgi complexes, microtubules, glycogen granules and membrane-delimited dense bodies or gliosomes. In Melanoplus differentialis the gliosomes contain acid phosphatase and are therefore equivalent to lysosomes (LANE, 1968). Mitochondria are also present in the glioplasm but are fewer in number compared to the perineurium. The glial nuclei, like those of the perineurial cells, contain dense clumps of chromatin material, particularly in the vicinity of the nuclear envelope, and a prominent nucleolus. They occasionally migrate from their more normal position beneath the perineurium and come to lie between the perikarya and the edge of the neuropile.

(Plate 63). Multiple stacks of narrow glial leaflets separate the perikaryal and neuropilar regions from one another.

Glial cytoplasm is poorly represented in the neuropile, though occasionally narrow glial elements can be detected interposed between the axon profiles (Plate 65).

The perikarya

The cortical perikarya are made conspicuous by their large, centrally positioned, ovoid or spheroid nuclei. The inner nuclear membrane is more than twice the thickness of the outer membrane (Plate 64), an observation also recorded for nuclei of the corpus cardiacum neurosecretory cells in Calliphora (NORMANN, 1965) and for perikaryal nuclei of the pars intercerebralis in Calliphora (BLOCH et al., 1966) and Locusta (ANSTEE, 1968). The nuclear sap contains a prominent nucleolus, dense clumps of chromatin material and scattered dense spheroid granules, ca. 90 nm in diameter (Plate 64). Granules of similar size, shape and electron density were observed by NORMANN (1965) in Calliphora in the perikaryal nuclei of the hypocerebral ganglion and in nuclei of the corpus cardiacum neurosecretory cells, where they occur in association with chromosomes. Mitochondria are plentiful and evenly distributed throughout the cytoplasm. The numerous randomly distributed profiles of the endoplasmic reticulum

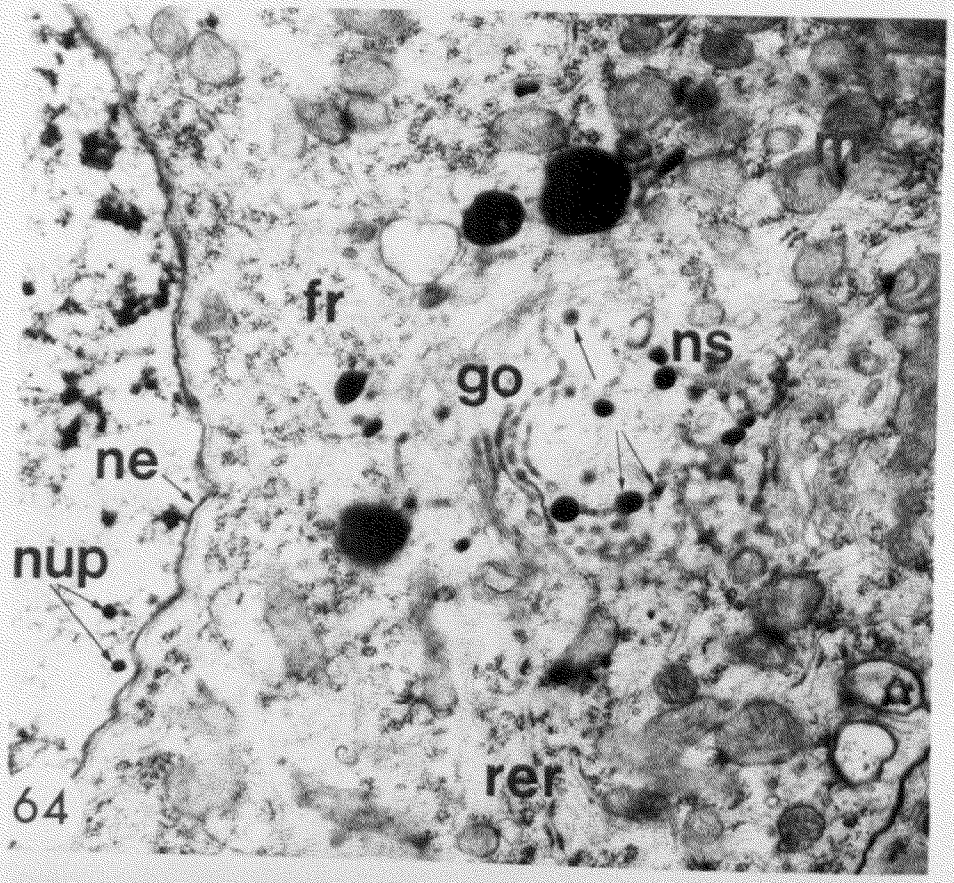
PLATE 64. Electron micrograph of part of a neurosecretory neurone of the hypocerebral ganglion. The Golgi complex (go) is actively manufacturing electron-opaque granules of neurosecretion (ns), some of which are in the process of being pinched off from the periphery (arrows). Profiles of rough endoplasmic reticulum (rer), mitochondria (m) and free ribosomes (fr) are frequently encountered in the perikaryal cytoplasm. Note the nuclear envelope with its thick inner and thin outer walls, and nuclear particles (np) in the nuclear sap.

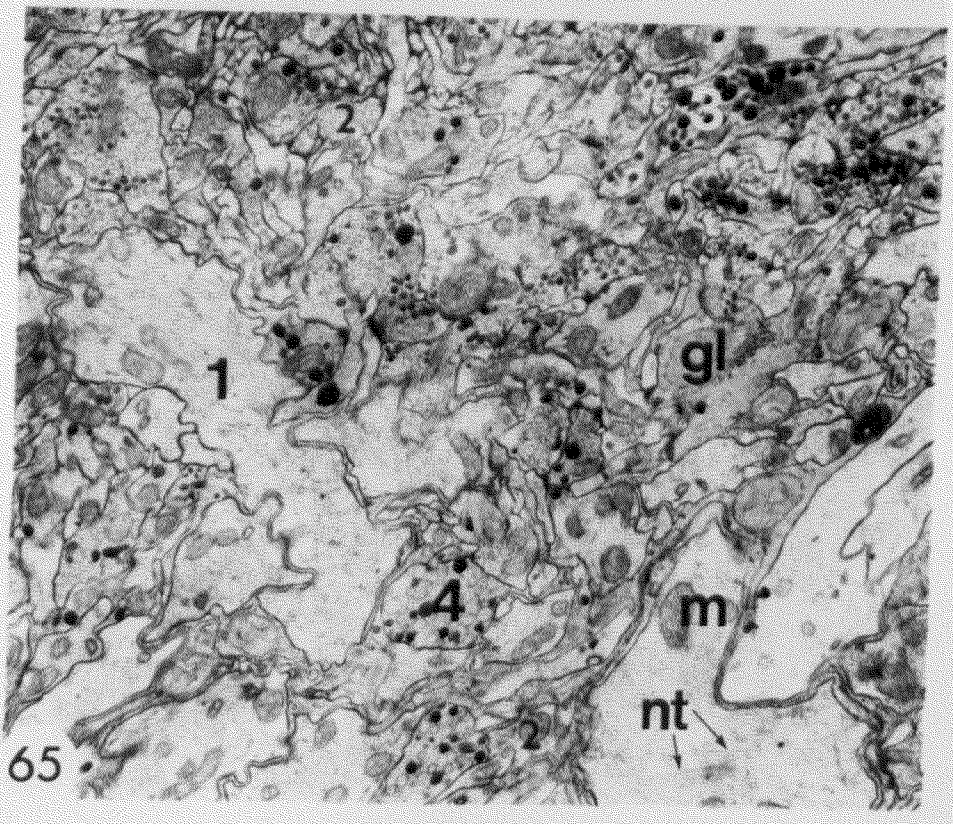
Magnification x 32,000.

PLATE 65. A survey field of the hypocerebral ganglion neuropile. Note the presence of four different axon types: 1 - containing mitochondria (m) and neurotubules (nt) but devoid of neurosecretory material; 2 - containing synaptic vesicles; 3 - containing electron-opaque neurosecretory granules only; 4 - containing electron-opaque and electron-lucent neurosecretory granules. Glial cytoplasm (gl).

Magnification x 15,000.

PLATES 64, 65





appear in the form of flattened membrane-limited cisternae of varying length and undulation to which are attached many ribosomes. Free ribosomes, which are sometimes aggregated into clusters, occur in abundance.

The Golgi apparatus is of special interest since it actively participates in the manufacture of neurosecretory granules, an observation first made by SCHARRER and BROWN (1961) in Lumbricus. This association between the Golgi complex and neurosecretion has since been described in many insect species (WILLEY and CHAPMAN, 1962; SCHARRER, 1963; NORMANN, 1965; BLOCH et al., 1966).

The perikaryal cytoplasm in the hypocerebral ganglion contains many Golgi complexes, a few of which are involved in the manufacture of neurosecretion. Each complex has the typical structure, consisting of stacks of flattened agranular cisternae in close association with vacuoles and vesicles of varying sizes. In favourable sections electron-dense material can be detected within the intracisternal membranes; this material becomes pinched off at the periphery of the cisternae to form membrane-bound granules. The membrane of the newly sequestered granule is usually quite obvious (Plate 64) but it later becomes obscured by the secretion product. Freshly elaborated neurosecretory granules measure 60-100 nm in diameter but electron-opaque granules ranging in diameter

up to a maximum of 500 nm can often be found in close proximity to the Golgi saccules (Plate 64). Electron-opaque neurosecretory granules were the only sort encountered in the neuronal perikarya.

The neuropile

The neuropile of the insect ganglion consists of a complex association of sensory, motor and internunciary axon processes together with gliocyte extensions (SMITH and TREHERNE, 1963). According to the nature of their axoplasmic inclusions bodies, five different 'Axon Types' can be recognised in the neuropile of the hypocerebral ganglion (Plates 65-68):

Axon Type 1 - Axon profiles containing neurotubules, ca. 20 nm in diameter, and mitochondria but no neurosecretory granules.

Axon Type 2 - Axon profiles also devoid of neurosecretion but partially or completely filled with small empty vesicles, ca. 30 nm in diamater; these are probably synaptic vesicles (SMITH and TREHERNE, 1963).

Axon Type 3 - Axon profiles containing electron-opaque neurosecretory granules, 140-200 nm in diamater.

Axon Type 4 - Axon profiles containing a heterogeneous population of neurosecretory granules, ranging in density from

PLATE 66. A section taken through the neuropile of the hypocerebral ganglion. The vast majority of the axons are charged with electron-opaque neurosecretory granules, but here and there can be found scattered type 4 axons containing both electron-opaque and electron-lucent granules.

Magnification x 17,500.

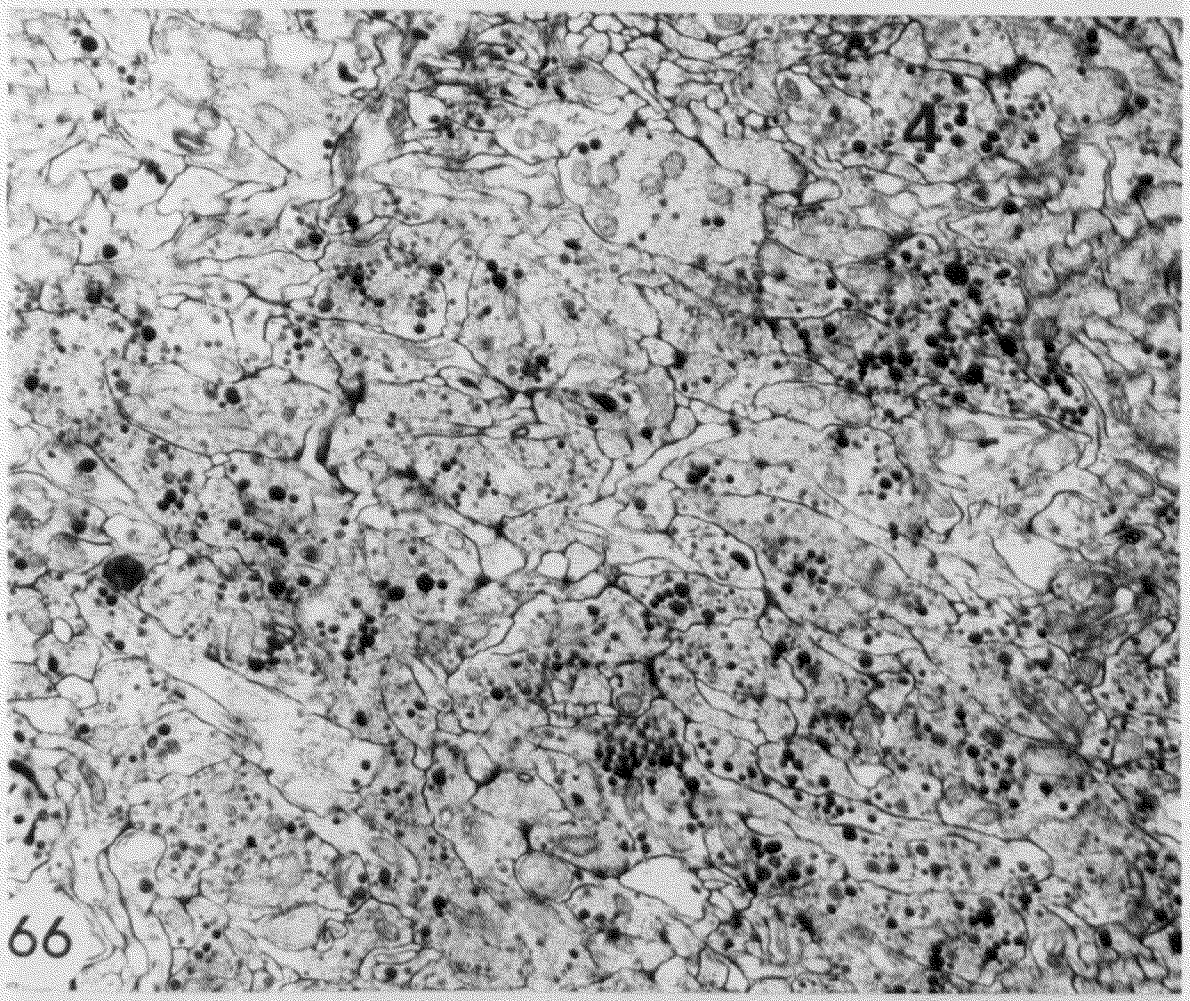
PLATE 67. A high power electron micrograph of the hypocerebral ganglion neuropile. Note adjacent type 4 axon profiles, one of which contains numerous small vesicles (v).

Magnification x 30,750.

PLATE 68. A high power electron micrograph of the hypocerebral ganglion neuropile. Note axon type 3, axon type 5, and glycogen granules (gy) contained within a narrow glial process.

Magnification x 20,000.

PLATES 66 – 68



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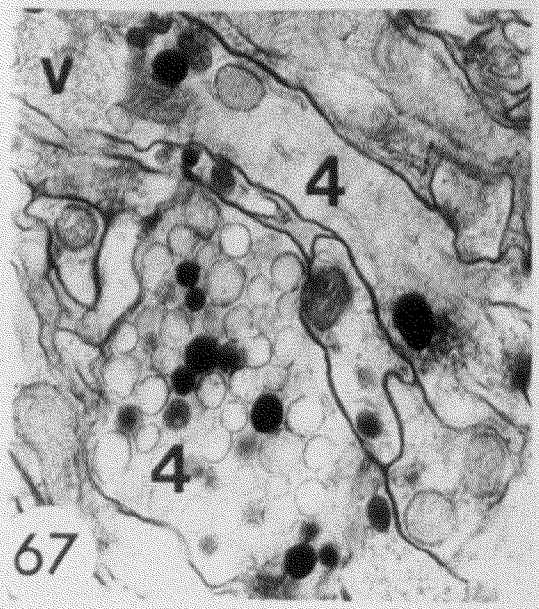
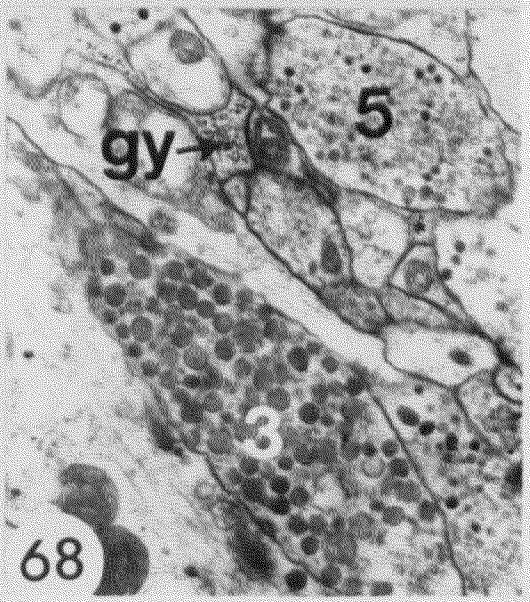


67



68

electron-opaque to electron-lucent, and measuring 140-200 nm in diameter.

Axon Type 5 - Axon profiles containing electron-opaque neurosecretory granules, 70-120 nm in diameter, and empty or partially filled vesicles, 50-60 nm in diameter.

Mitochondria and small vesicles, 30-45 nm in diameter, frequent Axon Types 3-5 (Plate 67) but neurotubules are less in evidence (Plate 68).

According to CAZAL et al. (1971) the only type of neurosecretory granule in the neuropile of the hypocerebral ganglion is the electron-opaque variety, ca. 100 nm in diameter. However, the granules in the electron micrograph presented by these authors measure nearer 150 nm in diameter, thus making them equivalent to the electron-opaque granules in Axon Type 3.

Ingluvial Ganglion

In its basic construction the ingluvial ganglion, of which there are two in Locusta, closely resembles the hypocerebral ganglion. Thus the medullary neuropile is almost completely surrounded by the cortical perikarya and both are invested with a nerve sheath consisting of an outer neural lamella and an inner perineurium. The glial cell bodies, the majority of which lie beneath the perineurium, give rise to

narrow cytoplasmic processes which encompass the perikarya and enter, to a limited extent, the neuropile.

The nerve sheath

The nerve sheath and perineurium do not differ significantly in their ultrastructural composition from these layers in the hypocerebral ganglion. Some of their more important features are depicted in Plate 69.

The perikarya

The cytoplasmic organisation of the perikarya mirrors that of the neurone cell bodies in the hypocerebral ganglion. Attention here is focused on the numerous Golgi complexes that are scattered throughout the cell body cytoplasm (Plate 70). Each complex is composed of a stack of flattened membrane-limited agranular cisternae, some of which often exhibit a beaded appearance. The majority of the Golgi bodies are involved in the manufacture of neurosecretory material which collects in a spheroid extrusion at the periphery of the cisterna and is finally separated off as a membrane-bound granule (Plate 71). Clear vesicles appear to be budded off from the ends of the cisternae in a similar manner to the granules and may also be formed by the breaking up of an

PLATE 69. Electron micrograph of the cortical region of the ingluvial ganglion. The upper portion of this field is occupied by the neural lamella (nl) in whose acellular matrix can be found collagen fibrils (cf) and tracheoles (tr). Immediately beneath the neural lamella is the perineurium (p). Note the system of intercellular clefts (icc) and intercellular spaces (ics), which eventually open out at the base of the neural lamella (arrows). Also the perineurial nucleus (pn) and mitochondria (m). Membranes of the glial system (gl) indent the cytoplasm of the perikaryon (pk) to form characteristic trophospongia (t). Gliosome (gs).

Magnification x 11,000.

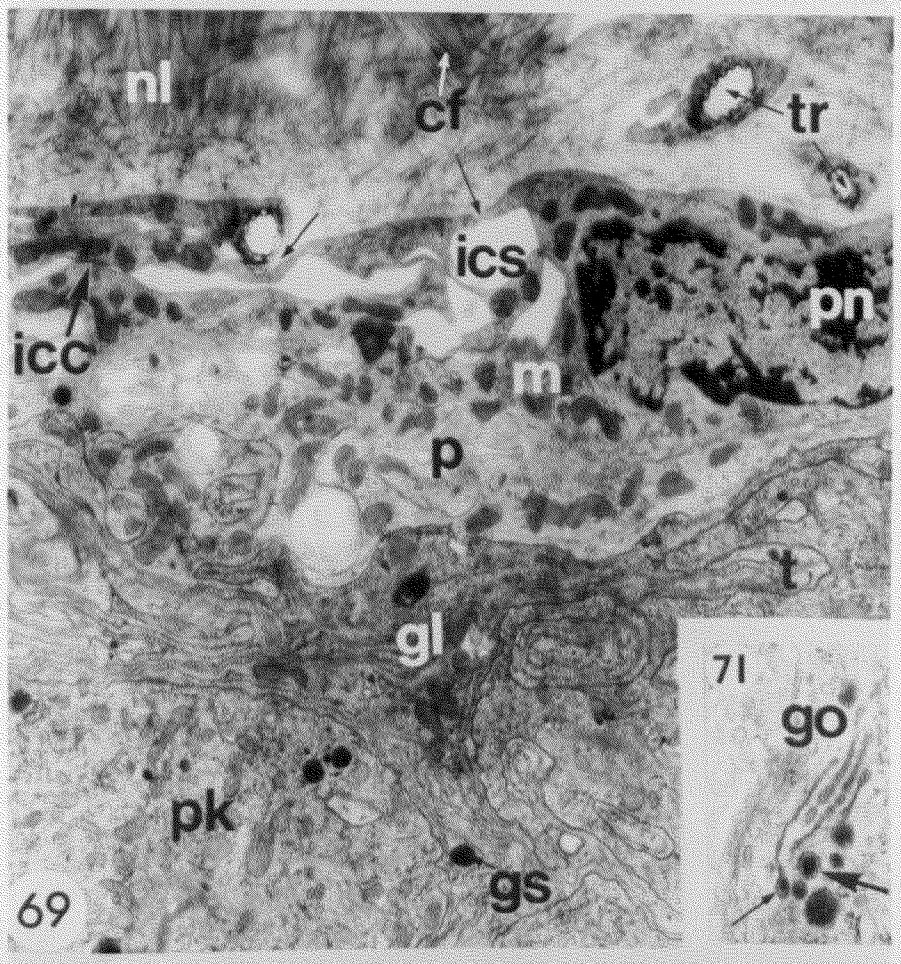
PLATE 70. A field illustrating some of the features of a neurosecretory neurone of the ingluvial ganglion. Note the numerous Golgi complexes (go), neurosecretory granules (ns), profiles of rough endoplasmic reticulum (rer), mitochondria (m), trophospongium (t) and perikaryal nucleus (pn). Some Golgi cisternae are breaking up to form small vesicles (vs).

Magnification x 15,700.

PLATE 71. High power electron micrograph of a Golgi complex (go) in the process of budding off a neurosecretory granule (small arrow). The large arrow indicates a freshly elaborated granule.

Magnification x 27,000.

PLATES 69 – 71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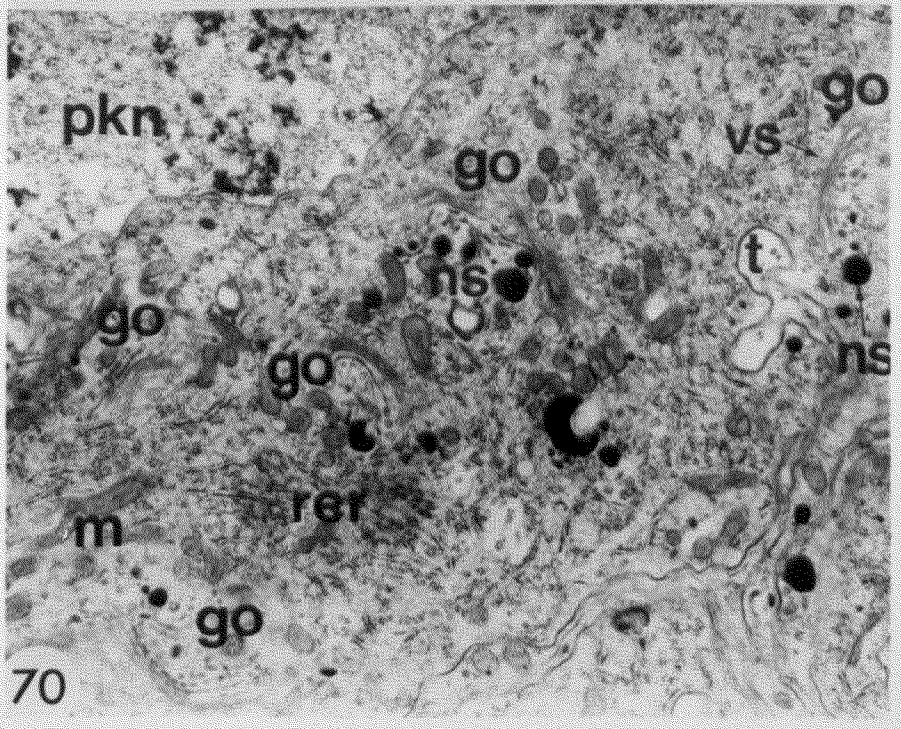
cf
tr
ics
pn
icc
m
p
t
gl
pk
gs
69
71
go


pkn
go
vs
go
t
ns
go
go
ns
rer
m
go
70

entire cisterna into smaller fractions which then round off to form vesicles. Freshly elaborated neurosecretory granules measure 70-100 nm in diameter, but a whole range of granule sizes up to a maximum diameter of 500-600 nm are present in the vicinity of the Golgi apparatus. The perikarya, in common with those in the hypocerebral ganglion, contain only the electron-opaque variety of neurosecretory granule.

The neuropile

As in the hypocerebral ganglion a number of different Axon Types can be recognised in each sectioned field of the ingluvial ganglion neuropile (Plates 72-74):

Axon Types 1-5 - These profiles correspond exactly to Axon Types 1-5 in the hypocerebral ganglion neuropile.

Axon Type 6 - Axon profiles containing electron-opaque neurosecretory granules, 200-300 nm in diameter.

Axon Type 7 - Axon profiles containing a heterogeneous population of neurosecretory granules which range in density from electron-opaque to electron-lucent and measure 200-300 nm in diameter. These were only very occasionally encountered.

Mitochondria and small empty vesicles, 30-50 nm in diameter, can often be found in Axon Types 3-7 but neurotubules are less obvious (Plates 73 and 74). In Plate 74 an electron-opaque granule appears to be in the process of budding off a

PLATE 72. A survey field of the ingluvial ganglion neuropile. Note the presence of four different axon types: 1 - containing neurotubules (nt) and mitochondria (m) but devoid of neurosecretory material; 2 - containing synaptic vesicles; 3 - containing electron-opaque neurosecretory granules only; 4 - containing electron-opaque and electron-lucent neurosecretory granules. Glial process (gl).

Magnification x 9,200.

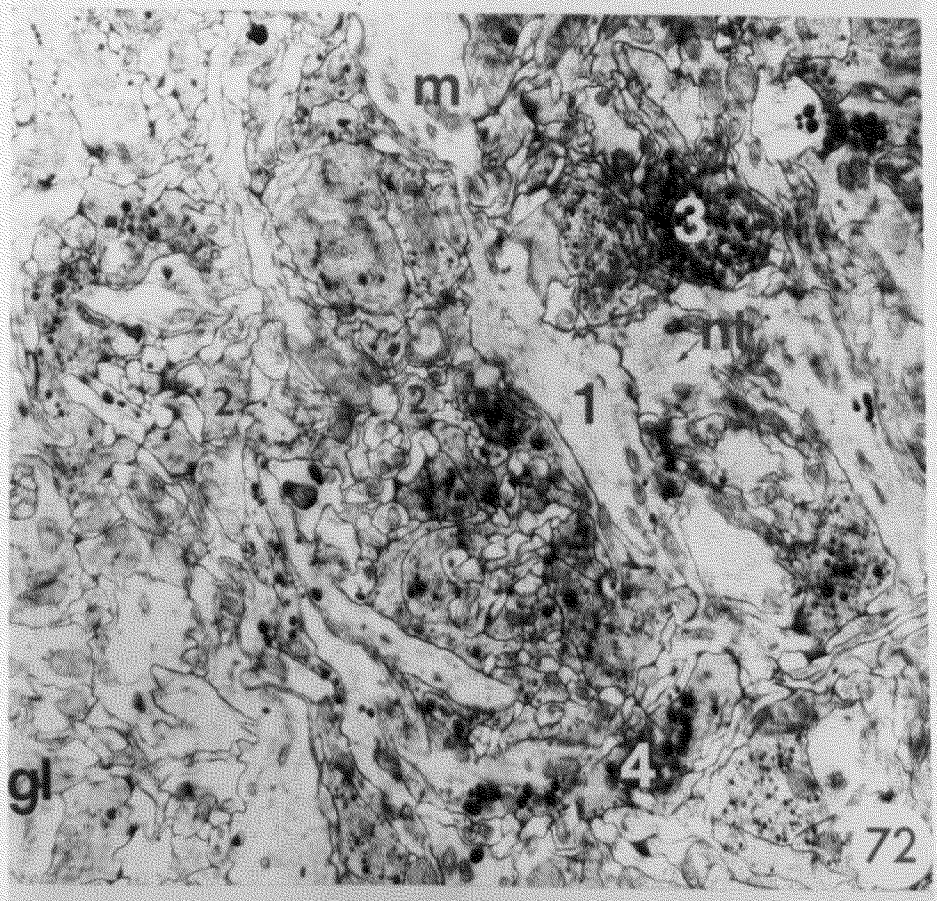
PLATE 73. A field of the ingluvial ganglion neuropile including profiles of axon types 2, 4 and 5.

Magnification x 14,100.

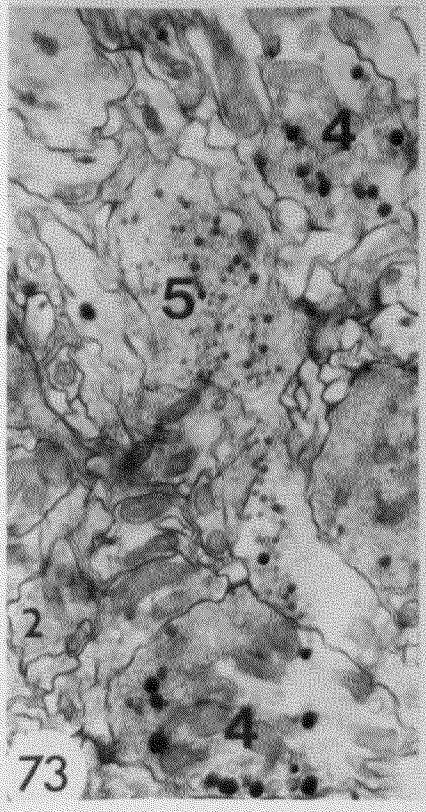
PLATE 74. A region of the ingluvial ganglion neuropile containing axon types 5 and 6. Towards the top of the field a neurosecretory granule appears to be in the process of budding off small vesicles (v).

Magnification x 16,700.

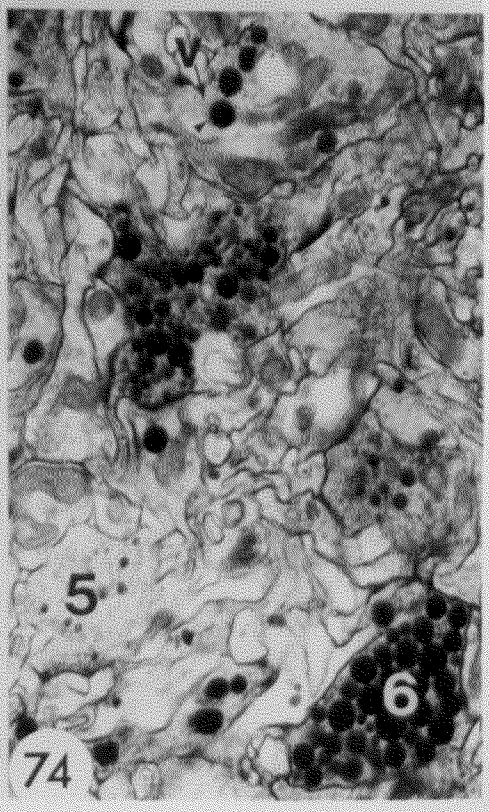
PLATES 72 – 74



72



73



74

small electron-lucent vesicle in a manner similar to that described by SCHARRER (1968) in Periplaneta.

The ingluvial ganglion neuropile in Blabera and Schistocerca (CHANUSSOT et al., 1969) contains two kinds of granule which often coexist in the same axon profile: (1) Electron-opaque granules, ca. 150 nm in diameter, but whose density and diameter show considerable variation. Such granules match those found in Axon Types 3 and 4 of the present work; (2) Vesicles with a diameter of 65 nm, the majority of which are clear with but a few containing electron-opaque material. The empty vesicles may correspond to those small empty vesicles frequently found in neurosecretory axon profiles in the Locusta ingluvial ganglion neuropile, although the vesicles of CHANUSSOT et al. are slightly larger. Non-neurosecretory axons (Axon Type 1) are also apparent in CHANUSSOT et al.'s electron micrograph of the neuropile in Blabera.

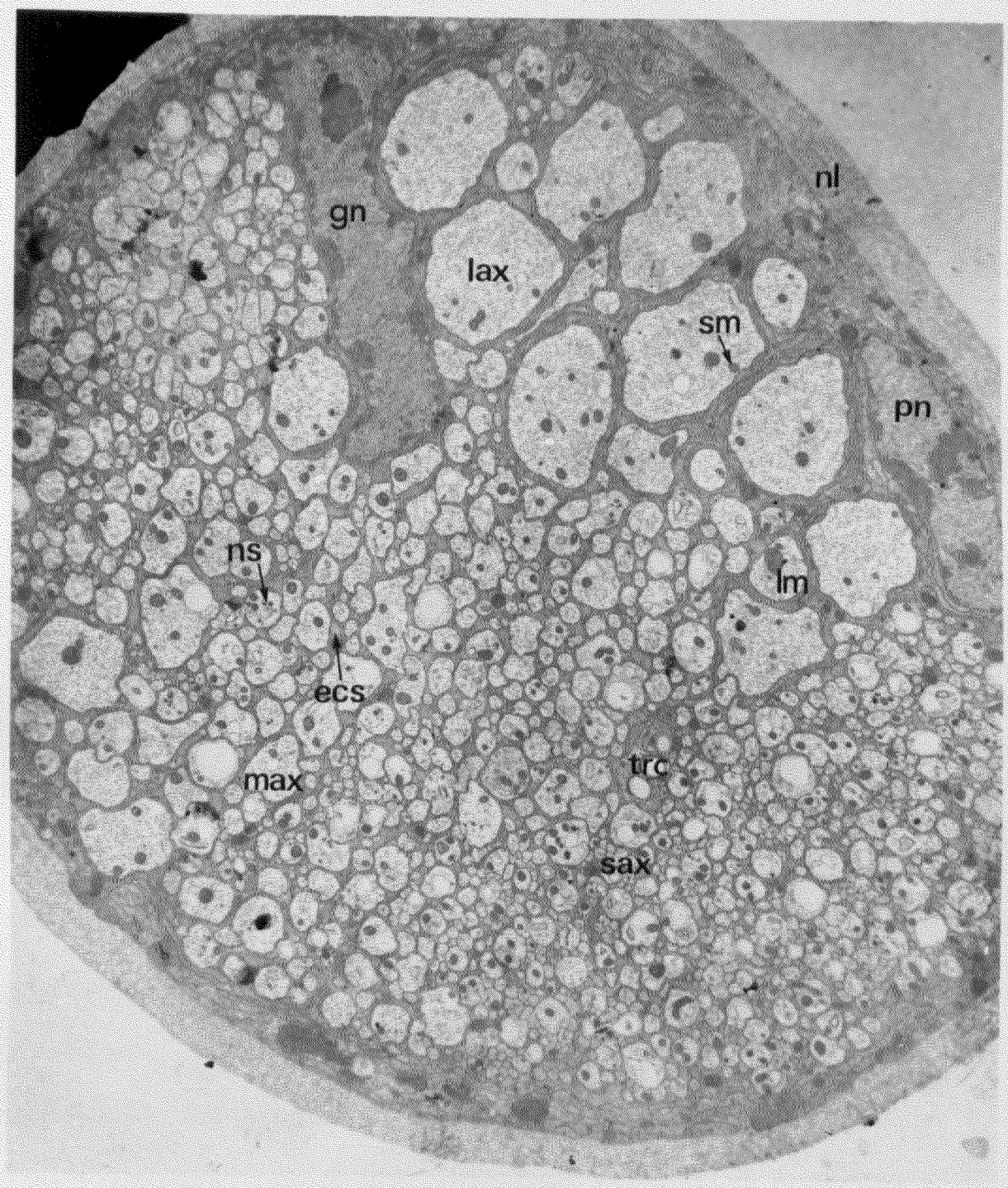
Frontal Connective

The two frontal connectives are almost circular in cross-section and one or other almost invariably accommodates a trachea which runs the length of the nerve between the centrally located axons. The nerve sheath (neural lamella + perineurium) overlies the glial cells in the cytoplasm of

PLATE 75. A transverse section of the frontal connective. The axon population is composed of a few large axons (lax) and numerous medium (max) and small axons (sax). One or more mesaxon turns invests each large axon, the space between the glial membranes periodically dilating to form large extracellular spaces (ecs). Neurosecretory material (ns) is located exclusively with the medium- and small-sized axon profiles. Note also the neural lamella (nl), perineurium nucleus (pn), gliocyte nucleus (gn), tracheolar cell (trc), small tubular mitochondria (sm) and large mitochondria (lm).

Magnification x 8,400.

PLATE 75



which are embedded the axon cylinders (Plate 75).

The nerve sheath

The neural lamella, which lies outside the perineurium, has the typical structure, being composed of a multidirectional meshwork of collagen-like fibrils embedded in an amorphous matrix.

Numerous tubular mitochondria occur in the cytoplasm of the perineurial cells and the sinuous lateral cell walls occasionally diverge to form intercellular spaces. Near their bases the lateral walls are held together by septate desmosomes (Plate 76). Compared to the hypocerebral and ingluvial ganglia, the system of lateral cell walls and intercellular spaces in the frontal connective is far less extensive. The nuclei of the perineurial cells are flattened and contain dense clumps of chromatin material. A few scattered Golgi complexes, consisting of several stacked saccules in close company with empty vesicles, can be found in the perineurial cytoplasm. Other cell organelles include microtubules, rough membrane-bound cisternae of the endoplasmic reticulum and free ribosomes. Vacuoles, sometimes containing membrane remnants, can also be detected in the cytoplasm (Plate 77). These probably represent the former site of dense granules

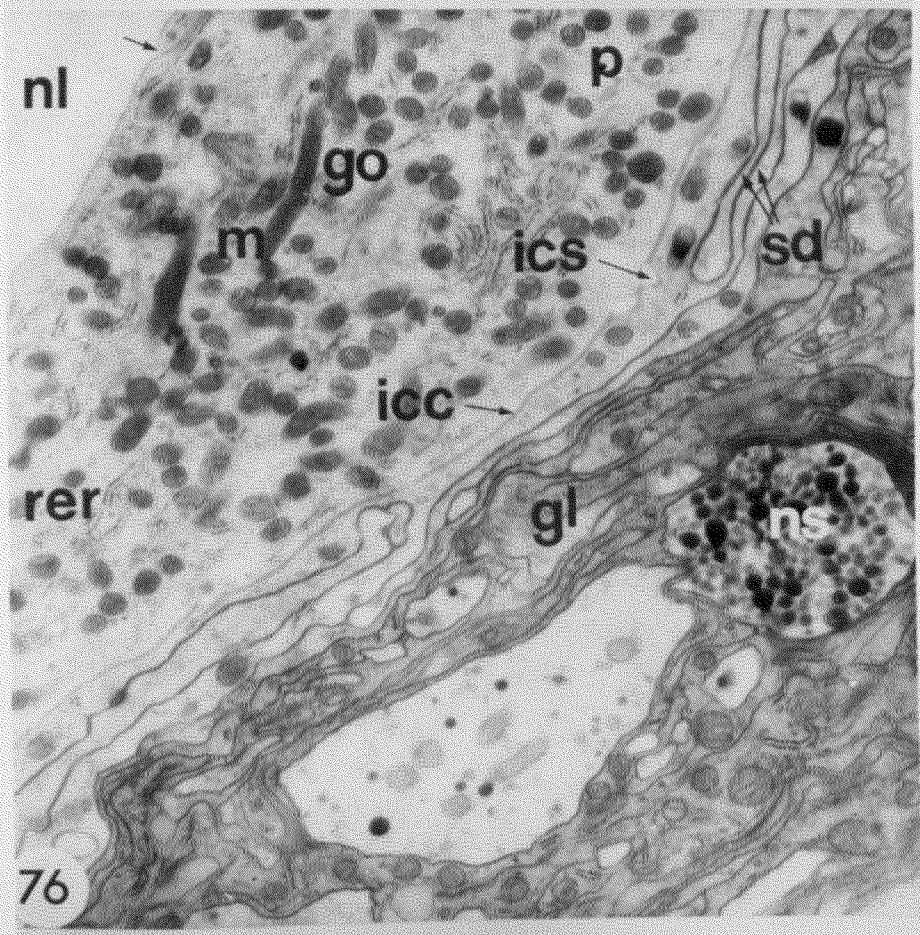
PLATE 76. A section taken through the nerve sheath of the frontal connective showing the large numbers of mitochondria (m) located in the perineurium (p). The lateral cell walls, which are held together by septate desmosomes (sd), frequently dilate to form intercellular spaces (ics). Note also the neural lamella (nl), profiles of rough endoplasmic reticulum (rer), Golgi complexes (go), glial processes (gl), axon packed full of neurosecretion (ns), and opening of the intercellular cleft (icc) immediately beneath the neural lamella (arrow).

Magnification x 17,100.

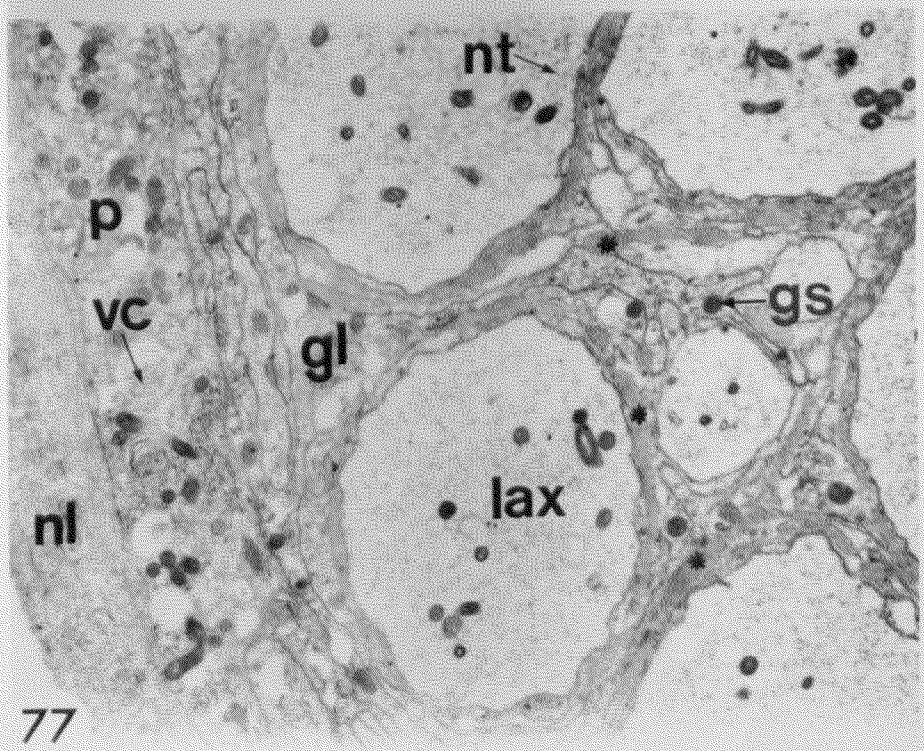
PLATE 77. A field including a group of large axon profiles (lax) within the frontal connective. The axons are ensheathed by glial cell processes (gl) the plasma membranes of which diverge periodically to delimit extracellular lacunae that become filled with an amorphous material (*). Note also neural lamella (nl), perineurium (p), neurotubules (nt), vacuole (vc) probably representing the former site of a dense granule, and gliosomes (gs).

Magnification x 15,000.

PLATES 76, 77



76



77

that have been extracted during glutaraldehyde fixation. The exact nature and function of these granules is obscure (LANE, 1968).

The neuroglia

The glial system is extensive, the cell bodies forming a layer beneath the perineurium and their narrow processes extending right to the centre of the frontal connective, between the axon profiles. The gliocyte nucleus is usually located peripherally but can sometimes be detected lying in the midst of the axon population. It is often indented to conform to the contours of nearby axon cylinders (Plate 75). Mitochondria, Golgi saccules and vesicles, rough membrane-bound cisternae of the endoplasmic reticulum, microtubules and gliosomes can be identified within the glial cytoplasm.

In the frontal connective the large axons are surrounded by one or more mesaxon turns. The space between the closely applied glial membranes, the extracellular cleft, periodically dilates to form large extracellular spaces which are filled with an electron-dense material, thus making them easily recognisable (Plates 75 and 77). According to ASHHURST (1961) and SMITH and TREHERNE (1963) this material is an acid mucopolysaccharide. Medium and small sized axons tend not to possess an individual glial membrane wrapping, but instead lie

free in the glial cytoplasm. They are, therefore, still isolated from one another except where they aggregate together to form 'axon clumps'. Such axon groupings share a common glial investment (Plate 79). The axolemma of the small axons is often in direct contact with the electron-dense material contained in the extracellular spaces (Plate 78).

The axon population

The transversely sectioned field of the fourth instar frontal connective presented in Plate 75 contains ca. 1200 axon profiles. Two classes of axon are recognised:

1. Large axons - these measure 1.7-2.6 μm in diameter and number 13. Their axoplasm contains many neurotubules as well as scattered mitochondria. The neurotubules, which are aligned in arrays roughly parallel to the limiting plasma membrane, resemble morphologically the microtubules in perineurial and glial cells. Both measure ca. 20 nm in diameter and appear, in transverse section, as minute empty vesicles. The precise function of the neurotubules is unknown although it is suggested that they may play some part in cytoplasmic flow or have a skeletal function (GRAY, 1970). Two distinct sizes of mitochondria occur in the large axons. In the smaller and more numerous type of mitochondrion the cristae

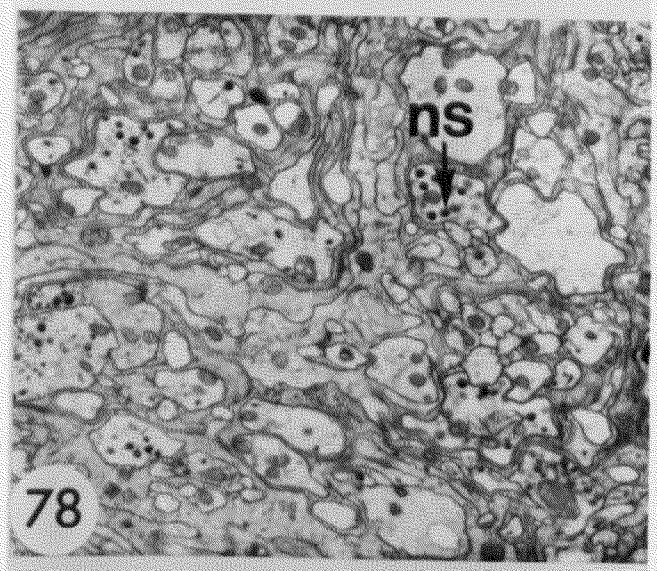
PLATES 78, 79. Sectioned fields of the frontal connective including groups of small axons (sax) containing neurosecretory material (ns).

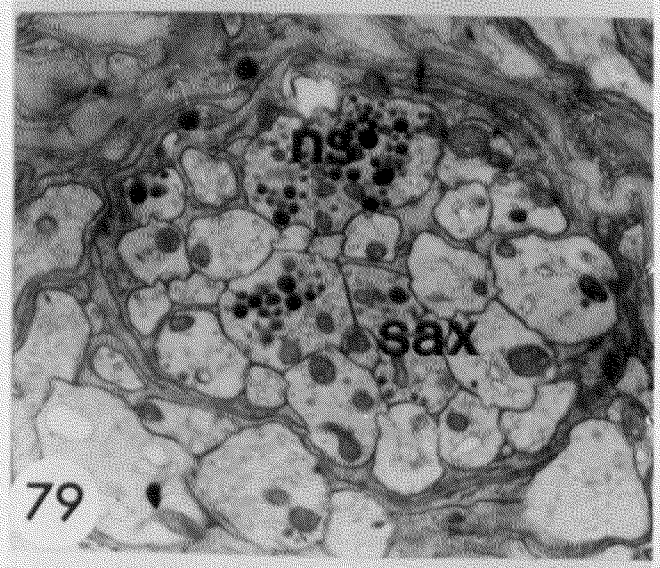
Magnifications x 11,600
x 17,600.

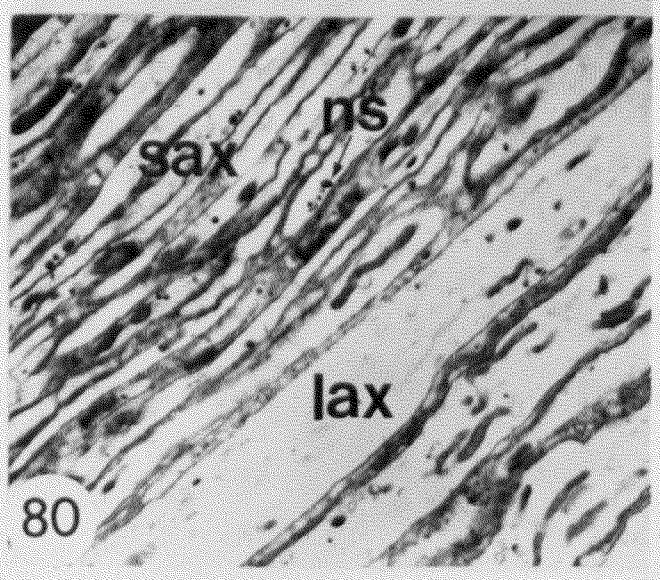
PLATE 80. Longitudinal section through part of the frontal connective axon population. Small axons (sax), large axons (lax), neurosecretion (ns).

Magnification x 10,500.

PLATES 78–80







appear either in the form of parallel lamellae or as concentric whorls. These types of arrangement are also adopted by cristae in the larger and rarer type of mitochondrion. A similar diversity in mitochondrial size and structure is also evident in the axons of the adult blowfly (OSBORNE, 1966).

2. Medium and small sized axons - these constitute the rest of the axon population and measure 0.06-1.3 μm in diameter. Neurotubules and small and large mitochondria can be found in medium and small sized axons together with a third type of axonal inclusion, the neurosecretory granule (Plates 75, 78, 79 and 80). By far the most predominant type of granule is the electron-opaque variety measuring 100-200 nm in diameter. A few grey granules of similar size range occasionally occur in the same axon as the electron opaque granules.

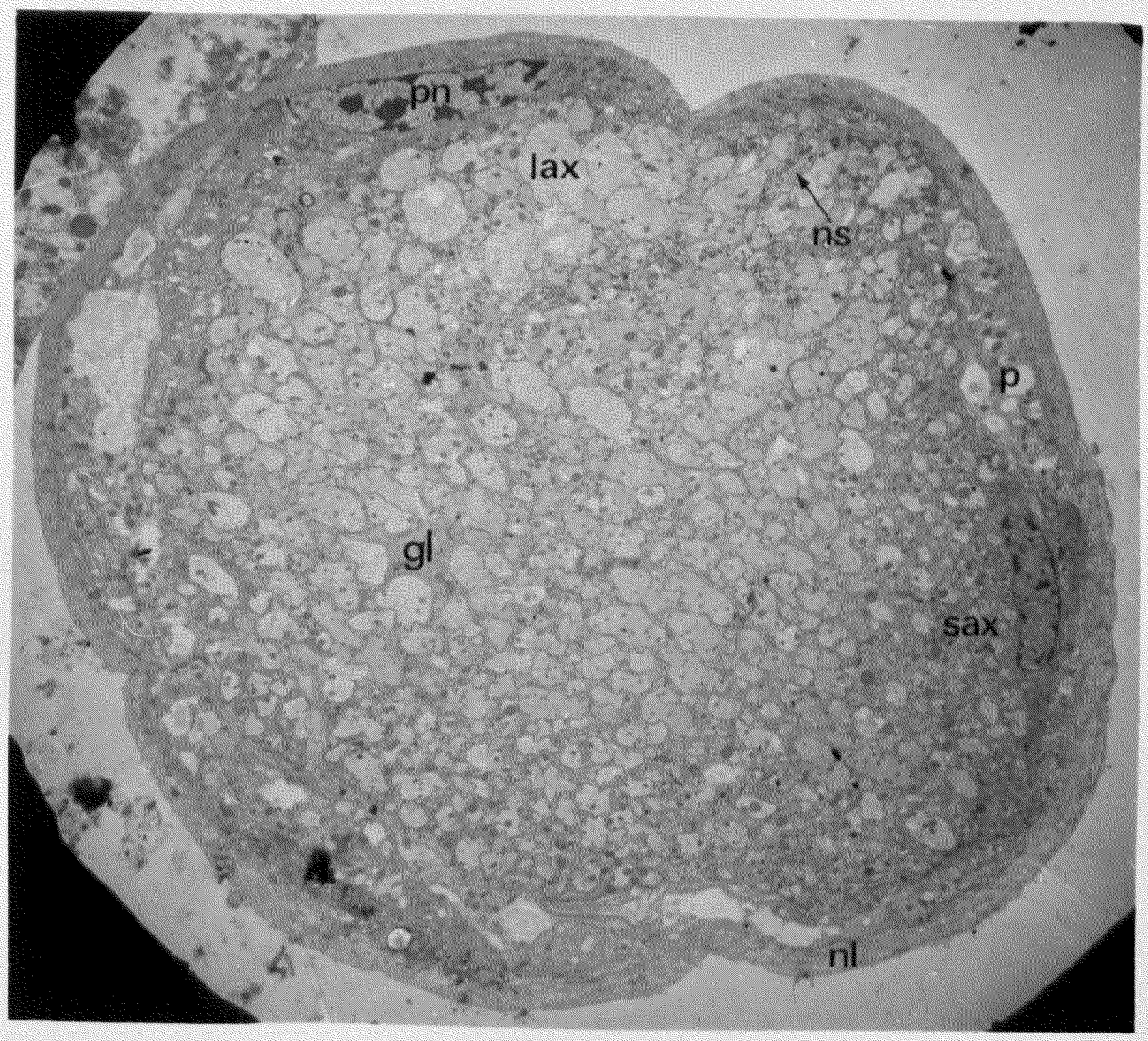
Recurrent Nerve

The recurrent nerve, which is a good deal thicker than the frontal connective, is approximately circular in cross-section. The centrally positioned axon population is invaded by the glial system and both are encapsulated by the nerve sheath (neural lamella + perineurium).

PLATE 81 A transverse section of the recurrent nerve. Note large axons (lax), small axons (sax), neurosecretion (ns), neural lamella (nl), perineurium (p), perineurium nucleus (pn) and glial process (gl).

Magnification x 4,100.

PLATE 81



The nerve sheath and neuroglia

In their ultrastructural organisation the neural lamella, perineurium and neuroglia do not differ significantly from these layers in the frontal connective. Some of their more important features are illustrated in Plates 81-84.

The axon population

At the level at which the section presented in Plate 81 is taken the fourth instar recurrent nerve contains approximately 2,200 axons in transverse profile, nearly twice the number counted in the frontal connective of the same instar (Plate 75).

Unlike the frontal connective, where a few large axons stand out from the rest of the axon population, there exists in the recurrent nerve a uniformly graded series of axon sizes, ranging from the smallest at 0.08 μm up to the largest at 2.7 μm in diameter. The disposition of the axons in the frontal connective and recurrent nerve is also different. Thus in the frontal connective the relatively few large axons tend to be grouped together near the edge of the axon population while in the recurrent nerve the larger axons may occupy a central position or display no organised grouping pattern whatsoever, as in Plate 81. The very small axons are

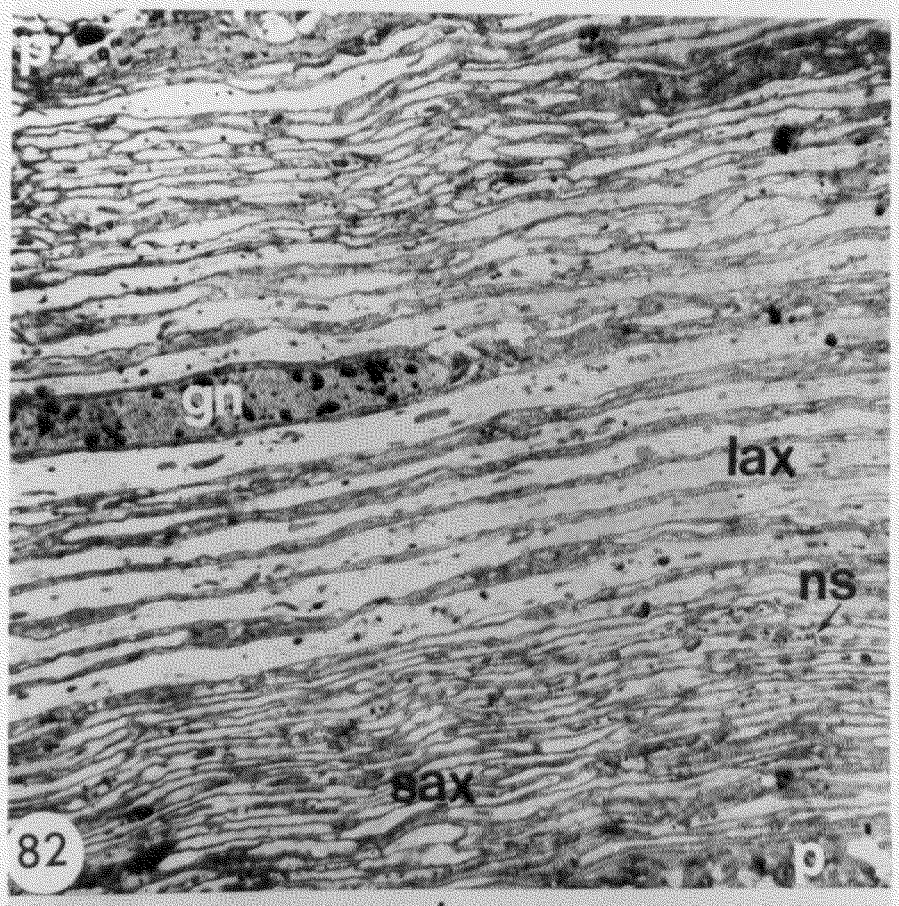
PLATE 82. A longitudinal section of the recurrent nerve. Note granules of neurosecretion in the small axons (sax), large axons (lax), perineurium (p) and gliocyte nucleus (gn).

Magnification x 6,000.

PLATE 83. A sectioned field of the recurrent nerve including a group of large axon profiles (lax) located immediately underneath the perineurium (p). Note the neural lamella (nl), perineurium nucleus (pn), mitochondria (m), rough endoplasmic reticulum (rer), glial cytoplasm (gl), neurotubules (nt), extracellular spaces filled with amorphous material (*), and opening of the intercellular cleft (icc) immediately below the neural lamella (arrow).

Magnification x 14,000.

PLATES 82, 83



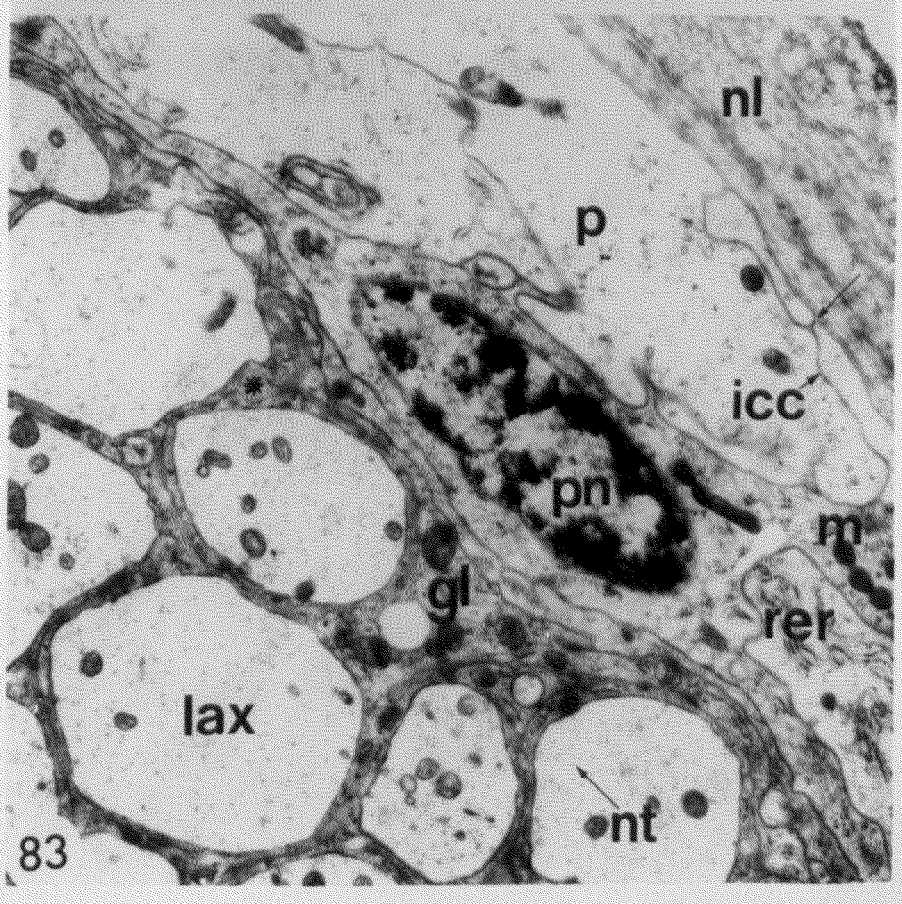


PLATE 84. A transverse section through a group of large (lax) and small axons (sax) in the recurrent nerve. Note neurosecretion (ns) in the small axons only, amorphous material in the extracellular spaces (*) and indented margin of large axon plasmalemma (arrow).

Magnification x 9,600.

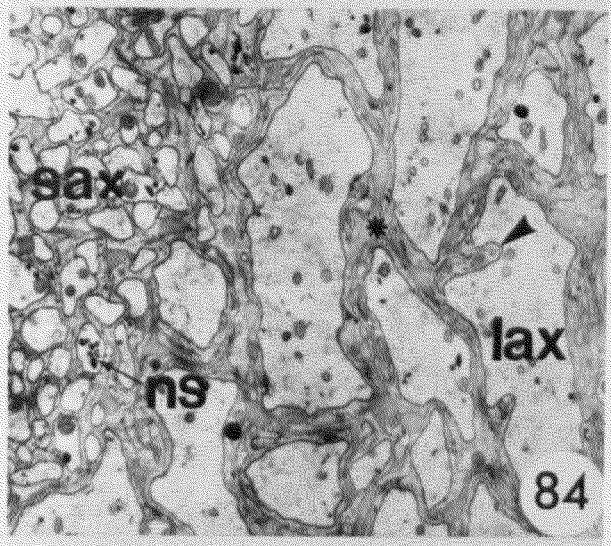
PLATE 85. A transverse section through a group of small axons (sax) containing neurosecretory material (ns). Several axon profiles contain synaptic vesicles (sv). Note also neural lamella (nl) and perineurium (p).

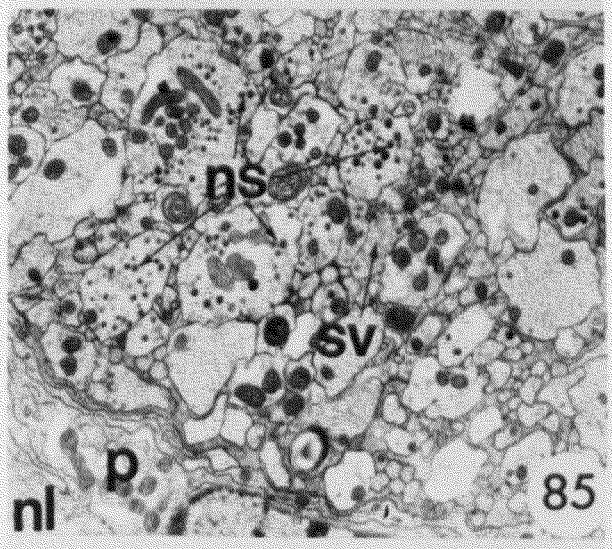
Magnification x 11,000.

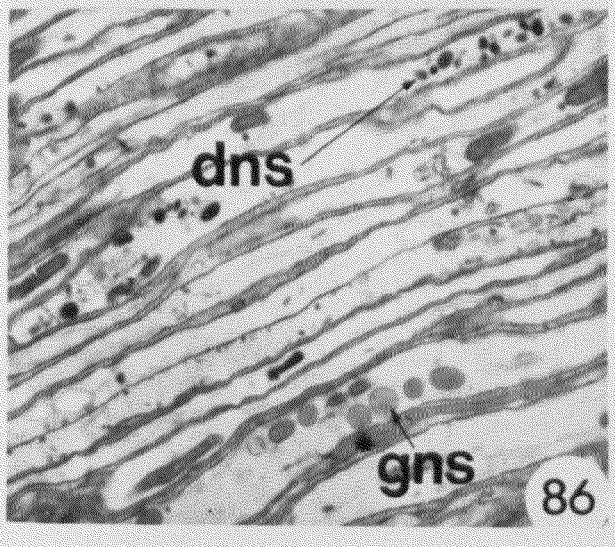
PLATE 86. A longitudinal section through a group of small neurosecretory axons in the recurrent nerve. Note dense granules of neurosecretion (dns), grey granules of neurosecretion (gns), and amorphous material in extracellular space (*).

Magnification x 15,400.

PLATES 84–86







invariably located close to the periphery of the axon population. The shape of the axons, particularly the large axons, in the recurrent nerve varies considerably, especially in transverse profile. In Plates 81, 83 and 85 the margins of the axons are frequently indented either by fellow axons or by glial cell cytoplasm, while in Plate 82 the axons are smooth in outline.

The larger axons, like those in the frontal connective, are non-neurosecretory. They contain neurotubules and small and large mitochondria whose cristae adopt a parallel lamellate or concentric whorl pattern of arrangement. Neurosecretory granules are found only in the medium and small sized axons which may also contain neurotubules and small and large mitochondria. The vast majority of granules are electron-opaque and measure 100-200 nm in diameter. Grey granules, 150-220 nm in diameter, are far less common (Plate 86). In Plate 85 several axon profiles contain a fourth type of axonal organelle, small clear vesicles 30-40 nm in diameter. These are almost certainly synaptic vesicles (SMITH, 1968).

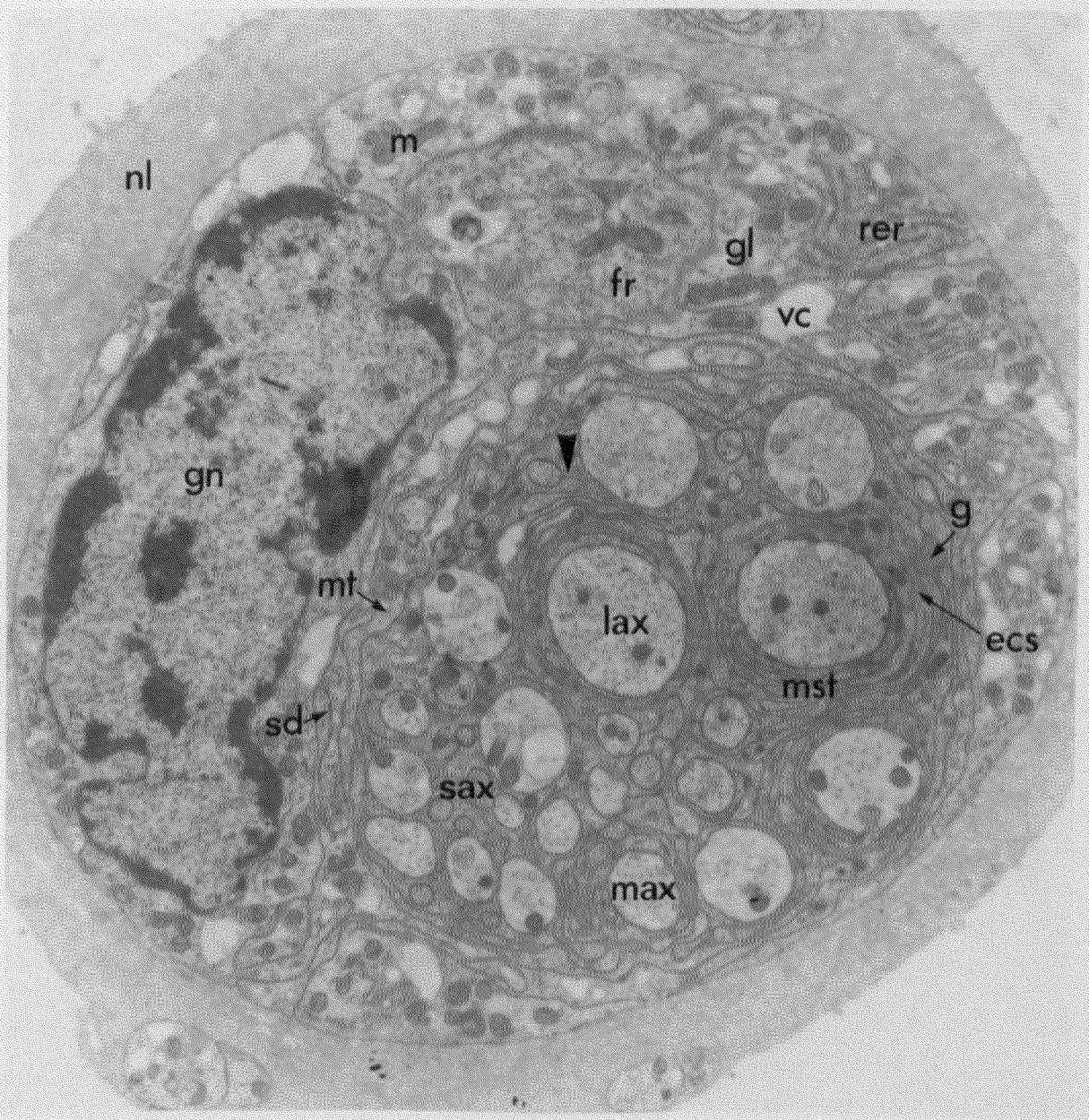
Posterior Pharyngeal Nerve

The posterior pharyngeal nerves in fourth instar nymphs were sectioned close to where they enter the frontal ganglion. In this position they are composed of an outer neural lamella

PLATE 87. A transverse section of the posterior pharyngeal nerve. Profiles of rough endoplasmic reticulum (rer), free ribosomes (fr), gliocyte nucleus (gn), microtubules (mt), gliosomes (g) and numerous mitochondria (m) can be detected within the glial cytoplasm (gl). Note that the cisterna of one rough endoplasmic reticulum profile is confluent with a large vacuole (vc). The axon population is composed of large (lax), medium (max) and small axons (sax). Each large axon is invested by one or more mesaxon turns (mst), the plasma membranes of the glial processes periodically diverging to form extracellular spaces (ecs). The amorphous material contained within these spaces may be in direct contact with the axolemma (arrow). Neural lamella (nl), septate desmosomes (sd).

Magnification x 12,900.

PLATE 87



inside of which lie the glial cells and axon cylinders (Plate 87).

The neural lamella and neuroglia

Tracheoles and isolated axons can be detected in the mottled background substance of the neural lamella as well as the usual collagen-like fibrils.

In the posterior pharyngeal nerve it is not possible to distinguish between perineurial cells and glial cells. This is also the case in small peripheral nerves in other insects (EDWARDS et al., 1958a,b, HESS, 1958; WIGGLESWORTH, 1959). The cells lying between the neural lamella and the axon population in the posterior pharyngeal nerve are considered to be glial cells. The glioplasm of these cells contains profiles of rough endoplasmic reticulum, a few scattered Golgi complexes, many microtubules, free ribosomes, gliosomes, mitochondria and a large gliocyte nucleus. Vacuoles of various shapes and sizes are also present and these, one must assume, were filled with material that has been removed during the cellular fixation process. One such vacuole is confluent with the cisterna of a rough endoplasmic reticulum profile (Plate 87). Contiguous glial cell membranes are often held together by septate desmosomes.

The larger axons in the posterior pharyngeal nerve enjoy at least one mesaxon fold while the rest are partially surrounded by glial membranes or lie free in the glioplasm. The closely-applied glial membranes frequently dilate to form larger extracellular spaces which contain an electron-dense substance similar to that found in the glial system surrounding the axons in the frontal connective and recurrent nerve (Plate 87).

The axon population

The transversely sectioned field of the fourth instar posterior pharyngeal nerve presented in Plate 87 contains 32 axon profiles which range in diameter from 0.2-1.7 μm. Their axoplasm contains neurotubules and many small and a few large mitochondria whose cristae adopt the now familiar parallel lamellate or concentric whorl pattern of arrangement.

A few sections also included fine branches of the posterior pharyngeal nerve (Plates 88 and 89). In these the perineurium is entirely absent and the scattered axons lie within the glial cytoplasm. Some of the axons contain electron-opaque neurosecretory droplets, measuring up to 220 nm in diameter, mingled with empty vesicles, 60-200 nm in diameter.

It is now clear that the nerve described by ANSTEE (1968) as being the recurrent nerve (Plates 5 and 6) is in fact the posterior pharyngeal nerve which of course runs very close and parallel to the recurrent nerve before entering the frontal ganglion.

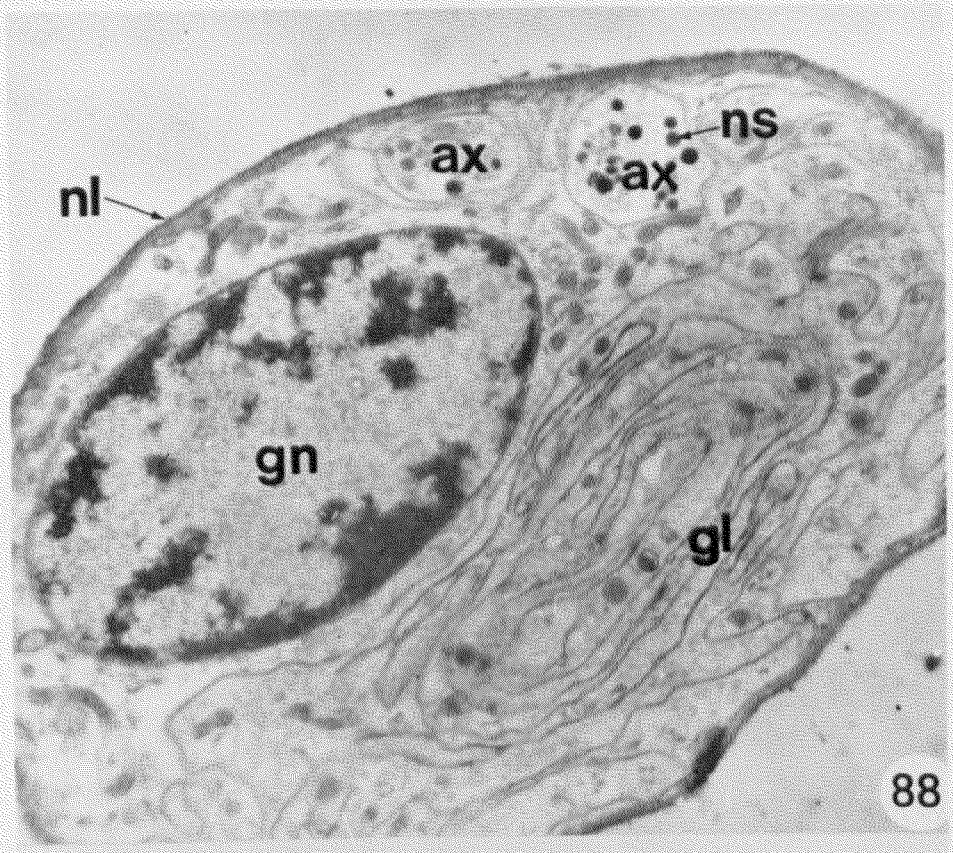
PLATE 88. Electron micrograph of a section through a branch of the posterior pharyngeal nerve. Neurosecretion (ns) is present in two axons (ax) located immediately below the neural lamella (nl). Glial system (gl), gliocyte nucleus (gn).

Magnification x 10,700.

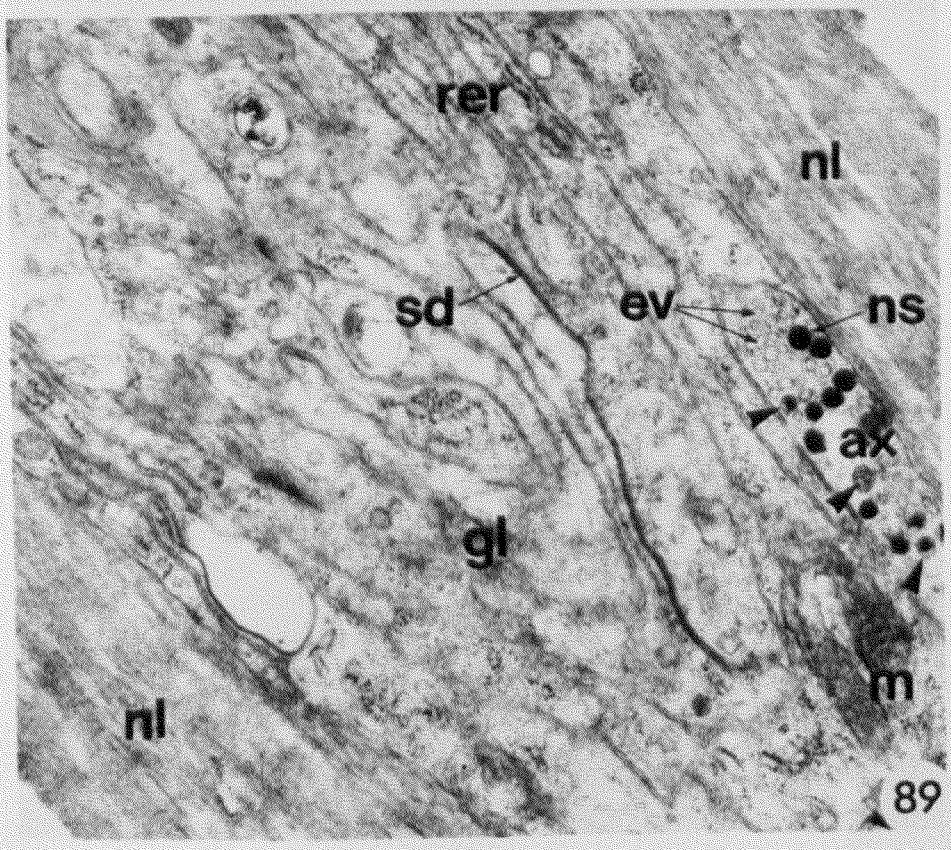
PLATE 89. Longitudinal section of a branch of the posterior pharyngeal nerve. Once again neurosecretory material (ns) can be detected in an axon (ax) lying immediately below the neural lamella (nl). The axon contains empty vesicles (ev) and part-empty vesicles (arrows). Note also the neural lamella (nl), glial system (gl), rough endoplasmic reticulum (rer), mitochondria (m) and septate desmosomes (sd).

Magnification x 30,000.

PLATES 88 – 89



88



89

SMITH and TREHERNE (1963) and ASHHURST (1968) review the functions of the neural lamella in insects. Its most obvious role is a mechanical one, that of maintaining the form of the ganglion or nerve, and in this it is helped by the collagen-like fibrils contained within the mucopolysaccharide matrix. At the same time the neural lamella remains permeable to ions and molecules that are exchanged between the haemolymph and the underlying perineurial cells.

MADDRELL and TREHERNE (1967) suggest that the perineurial cells influence axonal conduction by preserving within the ganglion or nerve an extracellular ionic balance that is very different from that in the haemolymph. According to these authors the long sinuous intercellular channels are employed in an osmoregulatory capacity, maintaining within their confined spaces solutions of high concentrations whose function it is to provide an osmotic 'buffer' between the haemolymph and the tissues underlying the cells of the perineurium. WIGGLESWORTH (1960) has shown histochemically that the perineurial cells take up nutrient precursors diffusing through the neural lamella from the haemolymph and pass them on to the underlying glial cells. Ion transport and food mobilization are processes requiring much metabolic energy and this accounts for the presence of the large numbers of mitochondria characteristically resident in perineurial cells. The perineurial cells are also held to be responsible for the secretion of the neural lamella (SCHARRER, 1939; ASHHURST, 1965; LANE, 1968).

The trophic function of the neuroglia, in supplying glycogen and lipids to the neurones, has been demonstrated

by WIGGLESWORTH (1960) and TREHERNE (1960). In favourable sections glycogen granules could be detected in the glial fingers which indent the peripheral cytoplasm of the perikarya in the hypocerebral and ingluvial ganglia. Glycogen granules also occur in the glial cell bodies, in the narrow glial processes entering the neuropile, and in the glial lamellae separating the neurone cell bodies from the edge of the neuropile. Axo-somatic and axo-dendritic synapses, which are commonplace in the vertebrate nervous system do not occur in insects because of the unbroken glial investment around the perikarya (CAJAL and SANCHEZ, 1951; SMITH and TREHERNE, 1963). Synaptic contact within the neuropile is to a certain extent defined by the glial processes entering this region (SMITH and TREHERNE, 1963). The acid mucopolysaccharide present in the extracellular spaces of the glial system in peripheral nerves functions to restrict the movement of ions and molecules in these spaces and also forms a significant cation reservoir, thus maintaining a relatively high sodium environment at the axon surface (TREHERNE and MORETON, 1970).

The neurone cell bodies of the hypocerebral ganglion in 10-day-old adults contain less neurosecretion than the perikarya of either the ingluvial ganglion or the frontal ganglion (ANSTEE, 1968) in adults of a similar age. Interestingly enough NORMANN (1965) could find hardly any neurosecretory material in the perikarya of the blowfly hypocerebral ganglion.

The most conspicuous feature of the perikarya in the ingluvial ganglion, and to a lesser extent in the hypocerebral ganglion, is the presence within them of 'giant' granules of neurosecretion. Freshly elaborated neurosecretory granules measure 60-100 nm in diameter, but in close proximity to the Golgi apparatus can be found a whole range of granule sizes up to a maximum diameter of ca. 500 nm. Giant neurosecretory granules also occur in the neurone cell bodies of the frontal ganglion where they measure 350-500 nm in diameter (ANSTEE, 1968). The giant granules are presumably formed by aggregation of smaller granules and according to GIRARDIE and GIRARDIE (1967) they represent a neurosecretory storage mechanism. The perikarya of the hypocerebral and ingluvial ganglia, like those of the frontal ganglion (ANSTEE, 1968) and most other insect ganglia, contain only one class of neurosecretory granule, namely the electron-opaque variety. The intrinsic secretory perikarya of the corpora cardiaca in Leucophaea (SCHARRER, 1963) and the alfalfa weevil, Hypera postica (TOMBES and SMITH, 1970), on the other hand, manufacture neurosecretory droplets of varying electron opacity. Whether or not such granules contain different hormones is at present unknown.

Neurosecretory granules differing in size and electron opacity occupy many of the axon profiles within the neuropile of the hypocerebral and ingluvial ganglia. Other axon profiles are non-neurosecretory in nature. As mentioned earlier Axon Types 1 - 5 are common to both the hypocerebral and ingluvial ganglia. The frontal ganglion neuropile (ANSTEE, 1968) contains axon profiles corresponding

to Axon Types 1 - 4 , while in the neuropile of the ingluvial ganglion of Blabera and Schistocerca (CHANUSSOT et al., 1969) can be found profiles which match Axon Types 1 , 3 and 5 . The single sectioned field of the Carausius hypocerebral ganglion neuropile presented by SMITH (1968) contains axon profiles which correspond to Axon Types 1 - 4 . Adopting the neurosecretory axon classification scheme of KNOWLES (1965, 1967), SCHARRER (1968) and CHANUSSOT et al. (1969), Axon Types 3 , 4 , 6 and 7 are peptidergic (A-type), carrying neurosecretory products of a proteinaceous nature, and Axon Type 5 is aminergic (B-type), transporting neurosecretory products of a monoamine character.

The presence of electron-lucent granules in Axon Types 4 and 7 can be interpreted in a number of different ways. They may, for example, contain neurosecretory material different to that in the electron-opaque granules and which is dissolved out by the fixative. Alternatively, they may contain a different sort of neurosecretion which happens to be electron-lucent (SMITH and SMITH, 1966; SCHARRER, 1968). A third explanation, and one for which there exists a certain amount of ultrastructural evidence, is that advanced by SCHARRER (1963) and BLOCH et al. (1966). These authors suggest that the treatment of the tissue in preparation for electron microscopy may cause the electron-opaque granules to swell and burst their membranes. Alternatively, rupture may occur because some granules are more fragile than others. Either way, a gradual emptying of the granule content occurs which eventually leads to a complete loss of electron density. In Plate 67 a Type 4 axon contains several electron-lucent granules whose membranes appear to have been ruptured.

Axon Type 2 , which may well be another region of Axon Type 1 , contains vesicles whose dimensions are the same as those of synaptic vesicles. The possible mode of formation of such vesicles is discussed by DE ROBERTIS (1964), VOLLRATH (1969) and HUDDART (1971b). Small vesicles (ca. 30 nm in diameter) are frequently encountered in Axon Types 3 - 5 and may be synaptic in nature too. Other vesicles occurring in Axon Type 5 measure 50-60 nm in diameter and occasionally contain dense material. Vesicles of a similar type have been detected in the ingluvial ganglion neuropile of Blabera and Schistocerca by CHANUSSOT et al. (1969) and are thought to contain a primary catecholamine.

The frontal connective and posterior pharyngeal nerve in fourth instar nymphs are both composed of a few large axons and many smaller axons. The large axons are invariably supplied with a private glial sheath which enables them to conduct at a faster rate than the more poorly insulated smaller axons (HUDDART, 1971a). Almost certainly the large axons are motor and the smaller ones sensory or motor (WIGGLESWORTH, 1969; HUGHES, 1965; BLANEY and CHAPMAN, 1969; HUDDART, 1971a). It is prudent, however, to heed the warning of PEARSON et al. (1970) who point out the dangers of using axon diameter as the sole criterion of function. Electrophysiological recordings from both large and small axons are obviously needed to supplement the ultrastructural observations presented here.

In the recurrent nerve and frontal connective neurosecretory material can be found only in the smaller axons. The neurosecretory axons in these nerves, and in the fine branches of the posterior pharyngeal nerve, contain mainly

the electron-opaque class of granule, grey granules being only rarely encountered. The large non-neurosecretory axons present in all three nerves presumably correspond to those found in the neuropilar region of the frontal and hypocerebral ganglia.

Giant neurosecretory granules of the sort found in the neuronal perikarya of the frontal, hypocerebral and ingluvial ganglia do not occur in the neuropiles of these ganglia or in the axons of the three peripheral nerves. One must assume, therefore, that they are broken down into granules of smaller size, probably by lysosomes (GIRARDIE et GIRARDIE, 1967), prior to transport out of the perikaryon.

CHAPTER IV

DISCUSSION

The morphology of the anterior stomatogastric nervous system in Locusta is more complex than previous studies have indicated (ALBRECHT, 1953; CLARKE and LANGLEY, 1963b, c). For example, the posterior pharyngeal nerve branches profusely and links the frontal ganglion to many structures including the musculature of the pharynx and oesophagus, the recurrent nerve, the hypocerebral ganglion, the tritocerebrum, the corpus cardiacum, the nervus corporis cardiacum III (NCC III) and the nervus corporis allatum II. Information from sensory cells on the pharynx can, therefore, reach the tritocerebrum and corpora cardiaca independently of the frontal connectives, frontal ganglion, recurrent nerve and hypocerebral ganglion. The complex nerve pattern, offering as it does many alternative routes between the foregut and the neuroendocrine system, increases the insect's chances of being able to adjust to the effects of any particular operation and of continuing the growth and moulting cycle. The ability of locusts to adjust to various surgical procedures is well illustrated by the work of HASKELL and MORDUE (1969). These authors demonstrated a recovery in feeding in hoppers after palpal removal and showed that other receptors were able to assume the role formerly discharged by the palps.

They deduced a rearrangement of the nervous integration mechanism to allow information from these other receptors (antennae and A_3 receptors of the clypeo-labrum) to be utilized instead.

The neuropilar region of the hypocerebral and ingluvial ganglia contains many A-type and relatively few B-type (KNOWLES, 1965; SCHARRER and WEITZMAN, 1970) neurosecretory fibres, as well as a certain number of non-neurosecretory fibres. It therefore resembles the ingluvial ganglion neuropile in Schistocerca gregaria and Blabera craniifer (CHANUSSOT et al., 1969; CHANUSSOT, 1972). The large intrinsic perikarya of the hypocerebral and ingluvial ganglia, like those of the frontal ganglion (ANSTEE, 1968; CAZAL et al., 1971), manufacture only the electron-opaque class of neurosecretory granule. Such granules are widely distributed and occur not only in the perikarya but also in the neuropilar region of each ganglion, in the smaller sized axons of the frontal connectives and recurrent nerve, and in axons contained in fine branches of the posterior pharyngeal nerve. At the light microscope level STRONG (1966) has detected neurosecretory material in the inner and outer oesophageal nerves in Locusta and future ultrastructural investigation will almost certainly reveal neurosecretion to be present in other nerves of the locust stomatogastric nervous system. But what is the functional significance of this neurosecretion?

There is increasing evidence to support the view that biogenic amines are involved in the control of foregut

muscular activity (FREEMAN, 1966; COOK et al., 1969). In this context it is interesting to note that CHANUSSOT et al. (1969) have localised a biogenic amine (probably dopamine or noradrenaline) in the ingluvial ganglion neuropile and in one of the ganglionic nerves in both Schistocerca and Blabera. Further, these authors believe that the primary catecholamine, whatever its true identity may be, is associated with the B-type neurosecretory fibres, that is those axons containing granules and vesicles ca. 65 nm in diameter. A similar kind of relationship between biogenic amines and B-type neurosecretory fibres has been deduced in the beta lobes of Periplaneta americana (MANCINI and FRONTALI, 1970) and in various vertebrate nervous tissues (HOKFELT, 1968; TAXI, 1968). The corpora cardiaca in Leucophaea maderae contain a few B-fibres, and SCHARRER (1963, 1968) suggests that these fibres may regulate the release of neurohormone from adjacent peptidergic A-fibres.

The abundance of neurosecretion in the ingluvial ganglion neuropile has led CHANUSSOT and his colleagues to suppose that this ganglion functions as a neurohaemal organ, storing neurosecretory material in the neuropilar axons and releasing it into the interaxonal glial cell extensions. The hypocerebral and ingluvial ganglia neuropiles in Locusta are likewise charged with copious amounts of neurosecretion, but whereas in the ingluvial ganglion much of this material is elaborated in the intrinsic perikarya, in the hypocerebral ganglion the neurone cell bodies appear to contribute very little neurosecretion to the neuropilar store. As a general rule the glial cell elements were found to be poorly represented in

the hypocerebral and ingluvial ganglia neuropiles and "axone-cellule gliale" contacts of the sort described by CHANUSSOT et al. (1969) and CHANUSSOT (1972) were not readily apparent in the sections of Locusta material examined.

Neurohaemal release sites, such as occur on the medial nerve in Schistocerca (BRADY and MADDRELL, 1967) and on the nervi corporis allati II in Acheta domesticus (WEBER and GAUDE, 1971), were not encountered in any section of the frontal connective or recurrent nerve. Some of the fine branches of the posterior pharyngeal nerve did, however, contain neurosecretory axons which were located immediately below the neural lamella. Release of neurosecretion into the haemolymph might be taking place since characteristic empty vesicles (SCHARRER, 1968) were present in the axon together with undischarged neurosecretory granules. The source of this neurosecretory material remains open to question but it could not have been derived from the main posterior pharyngeal nerve trunk since this was found to be devoid of neurosecretion. The material may have come from the NCC III. According to RAABE (1963a, 1964) this nerve is made up of axons of the tritocerebral neurosecretory cells and it has been shown during the course of the present study that the posterior pharyngeal nerve is linked to the NCC III.

Nerves containing neurosecretion run to a variety of tissues in insects (for references see OSBORNE et al., 1971) and it has been demonstrated by GOSBEE et al. (1968), WILKINS and MOTE (1970), and COOK and MILLIGAN (1972) that insect neurosecretory cells can conduct action potentials. By implicating the sensory cell bodies located on the surface

of the foregut one can speculate on the existence of reflex arcs in the stomatogastric nervous system. A nerve such as the inner oesophageal nerve, which is known to contain neurosecretion (STRONG, 1966), may have separate "neurosecretory" and "ordinary" motor nerve endings like, for example, the motor nerve of the ventral intersegmental muscles in Schistocerca (OSBORNE et al., 1971). The release of neurohormone and neurohumor at the nerve-muscle junction will be stimulated by the passage of spikes along the axons and the spike frequency will be determined, at least in part, by sensory imput reaching the neuronal perikarya from the surface of the foregut. BERN (1966) and SCHARRER (1969) believe that the function of neurosecretory innervation of tissues is to provide a more sustained stimulation than the classical neurohumoral type of activation which elicits responses of very short duration. The neurosecretory products may also have a trophic function, i.e. be involved in the growth and maintenance of the muscle tissue (OSBORNE et al., 1971).

ANSTEE (1968) has suggested that nervous impulses from the foregut sensory neurones may stimulate the release of neurosecretory material from the frontal ganglion perikarya. This material, travelling along the frontal connectives, then causes the release of neurosecretion from the medial neurosecretory cells and corpora cardiaca into the blood. The frontal connective neurosecretion may also stimulate the release of neurosecretion from the lateral neurosecretory cells of the pars intercerebralis. However, the rapid release of neurosecretory material from the neuroendocrine system, induced by feeding previously starved animals

(HIGHNAM et al., 1966; DOGRA and GILLOTT, 1971; HIGHNAM and WEST, 1971), can only be explained in terms of direct nervous stimulation. Therefore, the frontal connective neurosecretion, if it does play a stimulatory role, will be of importance over the long-term rather than the short-term period. The identification of synaptoid configurations (KNOWLES, 1967; SCHARRER, 1968), between frontal connective elements on the one hand and median or lateral neurosecretory elements on the other, would strengthen the above supposition that frontal connective neurosecretion does act as a neurochemical mediator. Some of the neurosecretory material carried in the frontal connectives may, in fact, find its way into the protocerebral neuropilar reservoir of HIGHNAM and WEST (1971). The assumption that neurosecretion in the frontal connective travels in the frontal ganglion $\longrightarrow$ tritocerebrum direction is supported by the results of preliminary ligature experiments, where a build up of neurosecretory material on the frontal ganglion side of the ligature has been demonstrated.

The remainder of Chapter V is devoted to a discussion on the effects of various surgical interruptions with the anterior stomatogastric nervous system on the processes of growth, new cuticle development and moulting.

The severance of the recurrent nerve either induces a hyperphagic-type response or has no adverse effect upon the insect apart from slightly lowering its growth rate. This

latter response is the only one reported by CLARKE and LANGLEY (1963b) for the operation in third instar animals, while the former response has previously been described in Phormia regina (see review by GELPERIN, 1971) and adult male Schistocerca (FRASER ROWELL, 1963). The induction of hyperphagia in Phormia depends on precisely where along its length the recurrent nerve is cut relative to a pair of stretch receptors contained in a link nerve running to the foregut surface (GELPERIN, 1967, 1971). Whether or not Locusta nymphs become hyperphagic may also be associated with the site of severance, and the posterior pharyngeal nerves appear to be in some way implicated in the determination of this response. Thus, fifth instar nymphs with both posterior pharyngeal nerves cut consume more food during the course of a single meal than control animals (BERNAYS and CHAPMAN, personal communication). These authors have also recently shown that the cutting of the recurrent nerve on its own has no effect upon the normal increase in resistance across the tips of the maxillary palps after the foregut has become filled with food, but that the cutting of the recurrent nerve plus the posterior pharyngeal nerves prevents this normal increase (BERNAYS and CHAPMAN, in press). A corollary of the latter observation is that the operated animals continue to respond to the presence of food and so become hyperphagic. In Phormia, Schistocerca and Locusta, then, hyperphagia is a response to surgical interference with a negative feedback pathway involved in inhibiting further feeding after filling of the foregut has been accomplished.

The cutting of the recurrent nerve, or its posterior extension beyond the hypocerebral ganglion, the oesophageal nerve (WILLEY, 1961), inhibits crop emptying over the short-term period (i.e. during the first 24 hr post-operative) in Periplaneta (DAVEY and TREHERNE, 1963) and Leucophaea (ENGELMANN, 1968), and over the long-term period in Leucophaea (SCHARRER, 1945; TAYLOR, 1969) and Melanoplus differentialis (DOGRA and EWEN, 1971). The short-term effects of the operation on crop emptying in Locusta were not investigated, but over the long-term period this process is not adversely affected. In those animals exhibiting a hyperphagic-type response almost the entire length of the gut become distended with food; this also happens in hyperphagic Phormia (EVANS and BARTON-BROWNE, 1960; GREEN, 1964; DETHIER, 1969).

In addition to its effects upon food consumption and food passage, severance of the recurrent nerve also induces the formation of tumour-like lesions in the foregut of many insect species (for literature review see TAYLOR, 1969), including Locusta (MATZ, 1961, 1963). TAYLOR considers that the invasive tumours in Leucophaea are an injury response to the overfull foregut but does not completely rule out the possibility of their being formed as a result of loss of neural innervation. HARKER (1963) suggests that the lesions are induced by humoral changes. Frontal ganglionectomy, an operation entailing severance of the recurrent nerve, also leads to the formation of tumours in the foregut of Locusta (ANSTEE, 1968; ROOME, 1968). Here the histological evidence suggests that, as in Leucophaea, they are caused by an overfull gut.

In accordance with CLARKE and LANGLEY (1963c) the cutting of one frontal connective has no effect upon the growth cycle in nymphal Locusta. On the other hand, the cutting of both frontal connectives (proximal to branches) does not, as these authors supposed, cause every operated animal to maintain a constant weight. The operation is a fairly straightforward one, yet when performed on animals of approximately the same age it yields a wide range of growth responses. Thus at one end of the growth spectrum lie those animals maintaining a constant weight and at the other those that more than double their operation weights. How, then, can this diverse effect upon growth be explained?

The site of connective severance was the same in every insect and care was taken not to damage other nerves during the operation. Surgical technique can, therefore, be ruled out as a cause of the variable growth response. There is evidence to indicate that the operation interferes with the process of crop emptying in acridids, and this in turn would influence growth. Thus ROOME (1968) reports that the cutting of both frontal connectives in third instar Locusta nymphs results in the majority of operated animals maintaining a constant weight, with but a few showing significant weight increases; the 'non-growers' invariably exhibiting distended crops at autopsy. The operation also adversely affects food passage out of the crop in adult Melanoplus (DOGRA and EWEN, 1971). By comparison, crop emptying in cockroaches is unaffected by the severance of both frontal connectives, at least over the short-term period (DAVEY and TREHERNE, 1963; ENGELMANN, 1968). The timing of the operation with regard to

prior feeding activity may, as ENGELMANN found following recurrent nerve severance in Leucophaea, be an important factor in determining the rate at which the crop empties. This implies that an insect with a full foregut at the time of the operation adjusts less well than one with an empty foregut. Such an idea is easy enough to test experimentally.

According to CLARKE and LANGLEY (1963c) and ANSTEE (1968) the frontal connectives play a role in stimulating the release of neurosecretion from the neuroendocrine system. The continued growth of some animals after the cutting of both frontal connectives distal to their branches provides clear evidence of the involvement of other nervous pathways in the control of neurosecretory release.

Severance of the anterior, median and posterior pharyngeal nerves has the opposite effect upon the growth cycle to that reported by CLARKE and LANGLEY (1963c) for third instars. Operated fifth instar nymphs exhibit twofold or more increases in weight and display an overall growth rate that is only slightly lower than that of the controls. There are two possible explanations for the discrepancy. The first of these assumes that the stomatogastric nervous system in third instar locusts is less extensive than in fifth instars, implying that the former group are neuromorphologically less well equipped to adjust to the effects of the operation than the latter group. The second possibility is that CLARKE and LANGLEY inflicted more damage upon the anterior stomatogastric nervous system than mere pharyngeal nerve severance. The operation obviously needs to be repeated on a large sample of newly moulted third instar nymphs and its effect

upon growth tested against that reported here for fifth instar nymphs.

Thus far, then, none of the operations involving severance of the nerves associated with the frontal ganglion have been shown to completely inhibit the growth cycle in each and every operated animal. Neither does the operation involving severance of both frontal connectives plus the recurrent nerve completely block growth in every operated adult locust. It is clear that after any of the above operations suitable alternative nervous pathways are open to the insect by which sensory information from the foregut can reach the neuroendocrine system. Over the long-term period the frontal connectives may play a more important role in the control of crop emptying than the recurrent nerve; the neural control of this process is certainly worthy of further investigation. It remains to be discovered whether in those operated animals exhibiting true growth the lower maximum weights attained compared to the controls are due to a drop in food consumption or to less efficient utilization of ingested food, or both.

CLARKE and LANGLEY (1963b, c) found that frontal ganglionectomy in third instar Locusta nymphs led to an immediate cessation of growth in every operated animal. They concluded that the operation acts by blocking the synthesis and/or release of neurosecretory material from the neuroendocrine system (CLARKE and LANGLEY, 1963d) but is without effect upon the feeding behaviour ("the intake of food, the amount in the gut, and defaecation proceed normally", CLARKE and LANGLEY, 1963b). The effects of frontal ganglionectomy on protein metabolism and RNA synthesis in Locusta nymphs,

as reported by CLARKE and GILLOTT (1965, 1967a, b), are held to be the result of a lack of neurosecretory material in the haemolymph rather than a lack of food material in the midgut ("the operated locusts were observed to eat frequently and to defaecate often; no obvious differences between these and normal locusts were observed in this matter", CLARKE and GILLOTT, 1965). In the present study, however, normal feeding behaviour could not be correlated with the maintenance of a constant body weight in any frontal ganglionectomised fourth or fifth instar locust. Food accumulated in the foregut of those operated animals maintaining a constant weight and passed through the gut unhindered in those exhibiting true growth (twofold or more weight increase). The vast majority of the frontal ganglionectomised locusts of ROOME (1968) maintained a constant weight after the operation and displayed a distended foregut at autopsy. Frontal ganglionectomy has an adverse effect on food movement in other acridids (ROUSSEL, 1966; HIGHNAM et al., 1966; HILL et al., 1966; GILLOTT et al., 1970; DOGRA and EWEN, 1971), but in the coleopteran Necrophorus vespillo normal alimentation and body weight increase occur after the operation (ROUSSEL, 1966).

Foregut muscular activity in Locusta is unaffected by the removal of the frontal ganglion (ROOME, 1968), while in Schistocerca its normal pattern of contraction and relaxation is changed following the removal of the hypocerebral ganglion (CLARKE and GRENVILLE, 1960). In both species movements of the foregut cease altogether in the absence of the ingluvial ganglia. Of the three stomatogastric ganglia, then, the frontal ganglion seems to play the least important role in

the control of foregut muscular activity. In its absence the pharynx and oesophagus presumably come under the influence of the hypocerebral ganglion and the fine nerve network that links it to these regions of the foregut. The oesophagus anyway displays a certain amount of myogenic activity, as does also the anterior region of the crop (ROOME, 1968). Thus, rather than influencing the normal peristaltic activity of the foregut musculature as HIGHNAM et al. (1966) suggest, the operation of frontal ganglionectomy appears to be exerting its effect at the level of the crop/midgut junction.

The neural control of crop emptying is complex and is fully understood in a limited number of insects only (see reviews by DAVEY, 1964; GELPERIN, 1971). In Leucophaea ENGELMANN (1968) concludes that "the opening of the proventricular valve appears to be controlled by either the ingluvial or the proventricular ganglion but that superimposed on these may be a certain amount of control by the frontal ganglion". It is clear that in Locusta frontal ganglionectomy has a variable effect upon crop emptying, and in this respect it resembles the operation of both frontal connective severance.

Frontal ganglionectomy can also affect food intake and food utilization. ROOME (1968) provides quantitative measurements to show that food consumption in operated Locusta nymphs is reduced in comparison to the controls. This, however, is presumed not to be the case in operated adult Melanoplus who, though they display greatly distended foreguts at autopsy and produce much less faeces than the controls, feed at an apparently normal rate (GILLOTT et al., 1970). The foreguts of a fair proportion of the frontal ganglionectomised animals

in the present investigation contained only moderate amounts of food at autopsy. In a few such animals the daily level of faeces production was the same as that in the controls but the body weight increases were much lower. Here the operation has interfered with the mechanisms of food utilization, presumably at the level of neurosecretory release (LANGLEY, 1962; GILLOTT, 1965).

In the normally growing locust neurosecretory material released from the corpora cardiaca regulates both the protein content of the haemolymph and the protein synthetic activity of the fat body (HILL, 1962, 1965; OSBORNE et al., 1968). In third instar (CLARKE and LANGLEY, 1963d) and fourth and fifth instar (ANSTEE, 1968; CLARKE and ANSTEE, 1971b) Locusta nymphs neurosecretory material accumulates in the nervi corporis cardiaci interni (NCC I) following removal of the frontal ganglion. CLARKE and LANGLEY consider that the operation has no effect upon food passage. The operated animals of ANSTEE maintained a constant weight and although this author makes no direct comment on the subject of food passage, the development of invasive tumours in the foregut of some of his operated animals, concluded by him on histological grounds to be caused by an overfull foregut, strongly suggests that crop emptying in these particular animals was inhibited by the operation. The few frontal ganglionectomised animals of ROOME (1968) that exhibited appreciable weight increases contained NCC I which, like those of the control animals, were devoid of stainable material. Unfortunately, this author did not investigate the NCC I in those operated animals maintaining a constant weight and displaying distended foreguts at autopsy.

In frontal ganglionectomised adult female Schistocerca (HIGHNAM et al., 1966) neurosecretory material piles up in the NCC I within two days of the operation, its accumulation coinciding with that of undigested food in the foregut. HIGHNAM and his collaborators consider that the operation increases the rate of synthesis and transport of neurosecretory material but has no adverse affects upon its release.

Frontal ganglionectomy in adult female Melanoplus (GILLOTT et al., 1970) has the same effect on crop emptying and hence body weight increase as in adult female Schistocerca, but an opposite effect upon the type A neurosecretory cells whose synthetic abilities are reduced after the operation. As GILLOTT et al. point out, the operation may lead directly to reduced activity in the cerebral neurosecretory cells (CLARKE and LANGLEY, 1963b, c, d) or it may affect these cells indirectly, via semi-starvation (HIGHNAM et al., 1966; HILL et al., 1966).

Brief mention should be made here of the effects of frontal ganglionectomy on the activity of the corpora allata in the above three acridids. These glands in Locusta nymphs (CLARKE and ANSTEE, 1971b) and adults (STROND, 1966) maintaining a constant weight after the operation show a marked reduction in size and, according to CLARKE and ANSTEE, appear histologically inactive. On the other hand the few Locusta nymphs of ROOME (1968) that increase in weight after frontal ganglionectomy contain corpora allata which are of similar dimensions to those of control animals. The corpora allata in operated adult female Schistocerca (HIGHNAM et al., 1966) are smaller than in the controls. However, it has been shown

(see review by MORDUE et al., 1970) that the volume of the corpus allatum is not necessarily a true indication of its synthetic ability and ideally other parameters should be measured. In this context GILLOTT et al. (1970) found that both the volume and the synthetic ability (measured autoradiographically) of the corpora allata in adult female Melanoplus are unaffected by the removal of the frontal ganglion. DOGRA and EWEN (1971) have implicated the corpora allata of Melanoplus in the control of general protein synthesis, while in adult female Locusta MINKS (1967) considers that these glands induce the formation of specific vitellogenic proteins in the fat body.

From the available evidence it appears that the effects on growth of various surgical interferences with the anterior stomatogastric nervous system are mediated primarily at the level of the crop/midgut junction. The operation may also reduce the endocrine potential of the insect in such a way that although normal or near-normal amounts of food are passed through the gut they are not fully utilized and converted into body tissue. There is plenty of scope for future work here. An obvious priority is a histological and autoradiographical examination of the neuroendocrine systems in growing and non-growing operated animals coupled with quantitative measurements of food intake and faecal production.

The development of a new cuticle is unaffected by the operations of one frontal connective severance or anterior, median and posterior pharyngeal nerve severance. On the other

hand, the cutting of both frontal connectives or the removal of the frontal ganglion have a variable effect upon the moulting cycle, some animals developing a new cuticle, others not (see also CLARKE and LANGLEY, 1963c; ROOME, 1968; PENZLIN, 1971).

The release of neurosecretion from the corpora cardiaca is a vital step in the moulting cycle, the neurosecretory material stimulating the prothoracic glands to secrete a hormone that activates the epidermal cells which in turn secrete a new cuticle (see reviews by WIGGLESWORTH, 1964, 1970). In Locusta, as in Rhodnius (WIGGLESWORTH, 1934, 1964), the prothoracic glands need to be exposed to the brain hormone for a certain 'critical' period in order that the moulting cycle might proceed to completion (JOLY et al., 1956; STRICH-HALBWACHS, 1959; GIRARDIE, 1964). CLARKE and LANGLEY (1963d), CLARKE and GILLOTT (1967a) and CLARKE and ANSTEE (1971b) provide histological and physiological evidence that frontal ganglionectomy interferes with the release of neurosecretion from the corpora cardiaca in Locusta nymphs. The operation has no effect on new cuticle development when performed after the critical period (CLARKE and LANGLEY, 1963c). If, however, the operation is carried out during the critical period two factors will determine whether or not the moulting cycle proceeds to completion. First, the extent to which the prothoracic glands have been activated by neurosecretion at the time of the operation (CLARKE and LANGLEY, 1963a, d). Second, the rate at which neurosecretory material is released into the haemolymph after the operation. Individual variation is also likely to exist in the degree of

sensitivity of each prothoracic gland to neurosecretion, and in the ability of each insect to adjust to the effects of the operation and to utilise remaining intact nervous pathways for the release of neurosecretion.

Towards the end of the stadium the locust sets in motion a sequence of events which culminate in the process of ecdysis, during which the insect sheds the old cuticle and expands the underlying newly developed one to its full size. The cessation of feeding, the emptying of the gut of food, and the swallowing of air constitute the aforesaid sequence.

The cue for cessation of feeding is not known in any insect, but its effect must be to elevate the taste threshold to a level which causes the insect to become unresponsive to the presence of food. The mechanism involved in maintaining an elevated taste threshold level over the moulting period may be similar to that deduced by BERNAYS and CHAPMAN (in press) following a single feed. These authors conclude that the filling of the foregut stimulates, by way of nerves of the anterior stomatogastric nervous system, the release of a hormone from the storage lobes of the corpora cardiaca. This hormone acts on the terminal sensilla of the palps, causing them to close and so increase their resistance levels. Thus, immediately after the consumption of the last instar meal the palp tips will exhibit a high taste threshold level. At this time the insect has already decided to completely empty its gut of food and, as the X-ray studies revealed, the moment food starts to leave the foregut air begins to enter through the mouth.

In this way some degree of stretch in the foregut is maintained and this, according to the hypothesis of CLARKE and LANGLEY (1963d), stimulates the continued release of hormone from the corpora cardiaca, which reaches a high titre in the absence of any food absorption. The circulating hormone, in addition to stimulating the prothoracic glands as CLARKE and LANGLEY suggest, may also serve to maintain the palp tip resistance at a high level. After ecdysis the movements of the foregut become less strenuous, the amount of hormone released diminishes, and eventually the palp tip resistance level falls to a value low enough to permit resumption of feeding. HILL and GOLDSWORTHY (1968) have shown that in fourth and fifth instar Locusta nymphs the development of the new cuticle takes place during the last 24 hr or so of the stadium, when feeding activity has ceased. It is possible that one and same cue halts feeding and stimulates old cuticle resorption and new cuticle deposition.

Food can continue to move through the gut unhindered in some frontal ganglionectomised animals during the feeding period but these same operated animals are unable to empty the gut of food at the end of the stadium. This paradox can perhaps be best explained by assuming that the brain plays no major part in the control of food passage during the feeding period, but does play a significant role in initiating, and perhaps even controlling, the process of gut emptying prior to air swallowing. The cerebral influence is mediated primarily at the level of the frontal ganglion (via the frontal connectives), but possibly also at the level of the

hypocerebral ganglion (via the NCC I, STRONG, 1966a), which in turn influences the ingluvial ganglia (via the outer oesophageal nerves). The cutting of both frontal connectives does not prevent normal emptying of the foregut in the vast majority of those animals attempting to moult to the adult stage. Presumably after this operation the frontal ganglion receives cerebral information via the NCC I, hypocerebral ganglion, recurrent nerve pathway. Neither is emptying of the foregut hampered by the lack of a direct nervous connection between the frontal ganglion and the pharynx, following severance of the pharyngeal nerves. Clearly other nerve pathways, such as the branches of the frontal connectives and the branches of the recurrent nerve, are relaying the motor information to the pharyngeal musculature.

Air swallowing at ecdysis is also under cerebral control. The operations of both frontal connective severance or anterior, median and posterior pharyngeal nerve severance interfere with the process of air swallowing and in all cases prevent a successful moult to the next stage. The moult fails because the operated animals are unable to swallow and/or retain sufficient air in the foregut to facilitate successful splitting of the old cuticle. Presumably the same nervous pathways as those mentioned above, in the context of food removal, carry motor information from the brain to the pharynx, thus permitting at least some air to enter the foregut following frontal connective or pharyngeal nerve severance. In addition some air will probably enter the foregut by passive diffusion.

LANGLEY (1962) found that operations involving the removal of the frontal ganglion, the cutting of both frontal connectives or the separation of the frontal ganglion from the surface of the pharynx caused the operated animals to lose water through the intersegmental membranes. This loss was thought to be due to the non-replacement of the proteinaceous components of the cuticle worn away as a result of the friction created by the intersegmental membranes rubbing against each other. CLARKE and GILLOTT (1967a) suggested that the low level of amino acids and proteins in the haemolymph of frontal ganglionectomised animals was the root cause for this water loss, the decrease in the concentration of these substances leading to a breakdown in water balance. Observations made during the present study, however, support the contention of ROOME (1968) that the 'water' is, in fact, regurgitated digestive fluid and grass juices which leak to the exterior from the mouth.

In accordance with LANGLEY the only operations causing 'leaking' are frontal ganglionectomy, both frontal connective severance, and pharyngeal nerve severance. The regurgitated fluid can be detected in the region of the mouth-parts and neck membrane as early as 2 days after the operation, timing that coincides with the provision of the first post-operative meal. ROOME suggests cerebral control of digestive fluid retention and this is the view taken here, the motor nerve pathways involved being the same as those controlling air intake and/or retention at ecdysis.

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ACKNOWLEDGEMENTS

The author wishes to express his gratitude for the guidance and encouragement of Dr. K.U. Clarke, who supervised this work.

He would also like to thank Professor E.J.W. Barrington for making available the facilities of the Zoology Department, without which this investigation would not have been possible; Mr. R. Searcy for his help with the photography, Mr. R. Gilder and his staff for providing readily available stocks of locusts, and Mrs. R.B. Richardson who typed this thesis.

Finally the author is indebted to Dr. R.F. Chapman for the use of library facilities at the Centre for Overseas Pest Research, London.

APPENDIX

The figures and summary tables presented in Chapter III were compiled from the raw data given on the ensuing pages.

All weights are expressed in milligrams; ages and times are given in days unless otherwise stated.

The following abbreviations have been used:

B.R. = before ringer.

A.R. = after ringer.

D = dead animal.

$\underline{976}$ = weight of newly moulted animal.

$\underset{\cdot}{9}\underset{\cdot}{0}\underset{\cdot}{1}$ = weight of animal at time of attempted moult.

* = displacement of labrum, caused by food accumulating in the preoral food cavity.

S.E. = standard error.

n.s. = values not significantly different (see 't' tests).

M, m. = male animal.

F, f. = female animal.

Schedule for Intra-vitam Methylene Blue Staining of Insect Nerves

(After STARK et al., 1969)

A. Solutions

1. Methylene blue stock solution

Dissolve 0.5 g methylene blue chloride in 100 ml distilled water. Heat the solution and stir until the solid is dissolved. Filter, cool and store.

2. Reduced (leuco) methylene blue solution

To 30 ml methylene blue stock solution add 6 drops concentrated HCl and 6 ml of 12% (w/v) Rongalit (Gurr Ltd.) solution. Stir and warm the mixture gently until it begins to turn a deep, dirty green colour, then remove from the heat and stir until colourless. Filter, after cooling, and store in the refrigerator in a tightly stoppered 40 ml bottle.

3. Fixative

8% (w/v) ammonium molybdate solution. Keep refrigerated and use cold.

B. Sequence

1. Inject 0.15 ml reduced methylene blue solution into the locust as described in Chapter II J and leave for 1 hr.

2. Open the insect under cold 8% ammonium molybdate (as previously described) and leave for 24 hr at 0°C.

3. Dissect out the appropriate portion of the nervous system and wash it thoroughly in several changes of distilled water.

4. Transfer to a drop of dilute glycerin albumen (Gurr Ltd.) solution on a coverslip. Remove excess fluid, orientate as desired and let partially dry in the air until the preparation is firmly attached to the coverslip. Keep the coverslip in a horizontal position for the remaining steps.

5. Dehydrate in two changes of tertiary butyl alcohol (1-2 hr each).

6. Clear in two changes of xylene (15 min each).

7. Apply the coverslip to a slide with synthetic resin (DPX).

Schedule for Preparation of Material for Examination Under the Electron Microscope

(After SMITH and SMITH, 1966; ANSTEE, 1968)

A. Preparation of Buffer and Fixatives

Note: Double distilled water and 'Analar' grade chemicals employed throughout. All solutions kept refrigerated at 0°C.

1. 0.1 M phosphate buffer, pH 7.4

Dissolve 15.6 g $NaH_2PO_4.2H_2O$ in 1 litre of distilled water (Solution A).

Dissolve 35.8 g $Na_2HPO_4.12H_2O$ in 1 litre of distilled water (Solution B).

To 190 ml Solution A add 810 ml Solution B to give 1 litre 0.1 M phosphate buffer, pH 7.4.

2. Addition of sucrose

To make 0.17 M sucrose solution in 0.1 M phosphate buffer, pH 7.4, dissolve 58.14 g sucrose in 1 litre of buffer.

To make 0.34 M sucrose solution in 0.1 M phosphate buffer, pH 7.4, dissolve 116.28 g sucrose in 1 litre of buffer.

3. 2.5% glutaraldehyde solution

A specially purified and stabilized 25% solution of glutaraldehyde for use in electron microscopy was obtained from TAAB laboratories. The 2.5% solution of glutaraldehyde used for fixation was prepared by adding 1 part of stock solution to 9 parts of 0.1 M phosphate buffer, pH 7.4, containing 0.17 M sucrose.

4. 1% OsO_4 solution

0.1 g OsO_4 was dissolved in 10 ml 0.1 M phosphate buffer, pH 7.4, containing 0.34 M sucrose. When not in use this solution was kept frozen.

B. Preparation of Araldite Monomer

CIBA Araldite epoxy resin CY212(M)	10 ml	Araldite monomer
CIBA Araldite hardener HY964	10 ml	
BDH Dibutyl phthalate (plasticiser)	0.8 ml	

To set monomer add:

CIBA Araldite accelerator DY064 0.45-0.50 ml

C. Fixation

1. Fix tissues for 2 hr at 0°C in ice-cold 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, containing 0.17 M sucrose.

2. Replace fixative with 0.1 M phosphate buffer, pH 7.4, containing 0.34 M sucrose and wash overnight. Dissect out the required tissue at this stage.

3. Wash again in fresh buffer solution.

4. Post-fix for 1 hr at 0°C in 1% OsO_4 in 0.1 M phosphate buffer, pH 7.4, containing 0.34 M sucrose.

5. Wash in two changes of buffer at 0°C (15 min each change).

D. Dehydration

6. Dehydrate at 0°C in 50%, 70% and 90% ethanol (30 min each change). Tissues may be left overnight in 70% ethanol.

7. Transfer to cold spectroscopically pure absolute ethanol containing 1% uranyl acetate, freshly prepared and Millipore filtered before use. Leave for 1 hr at room temperature.

8. Wash in fresh spectroscopically pure absolute ethanol for a further 1 hr at room temperature.

E. Embedding

9. Place in 25% Araldite monomer (made up in spectroscopically pure 100% ethanol) for 1 hr at room temperature.

10. Transfer to 50% and 75% ethanolic monomer solutions - 1 hr in each at room temperature.

11. Embed in 100% Araldite monomer for 1 day at 48°C.

12. Transfer tissues to Araldite monomer + accelerator for 1 day at room temperature.

13. Move into Araldite monomer + accelerator for a further 1 day at room temperature.

14. Transfer the material into dry, clean BEEM capsules which immediately prior to this have been filled with fresh Araldite monomer + accelerator. Polymerise in an oven at 90°C for 2 days. At the end of this time the blocks are ready for sectioning.

Note: Tissues requiring special orientation, e.g. nerves, were embedded in the lids of BEEM capsules.

TABLE I

Daily body weight changes of adult male and female Locusta used in Section I of Chapter III.

TABLE I (a) Operated controls

No.	Sex	Body weight (in mg) on Day: 1 (B.R.)	1 (A.R.)	2	3	4	5	6	7	8	9	10
1	M	1145	1190	1105	1170	1190	1315	1360	1515	1530	1630	1630
2	M	1035	1090	1035	1245	1165	1330	1405	1520	1490	1565	1600
3	M	1040	1090	1040	1250	1180	1210	1125	1360	1365	1440	1455
4	M	1070	1100	1040	1240	1070	1170	1220	1395	1390	1495	1440
5	M	1000	1030	990	1135	1090	1070	1160	1245	1380	1315	1380
6	M	920	955	925	960	875	995	1095	1245	1305	1255	1305
7	F	1375	1410	1360	1610	1575	1650	1700	1940	2080	2400	2380
8	F	1265	1310	1265	1435	1270	1460	1595	1820	1965	2300	2500
9	F	1170	1235	1180	1360	1390	1410	1455	1710	1790	1995	2030
10	F	1170	1220	1170	1405	1315	1310	1470	1680	1790	1925	2090
11	F	1255	1310	1240	1500	1460	1640	1660	2070	2295	2590	2615
12	F	1380	1415	1330	1605	1385	1485	1625	1910	2120	2310	2570

TABLE I (b) 1 Frontal connective cut

No.	Sex	Body weight (in mg) on Day: 1 (B.R.)	1 (A.R.)	2	3	4	5	6	7	8	9	10
1	M	1000	1085	1040	920	830	1040	1010	1190	1135	1255	1355
2	M	1140	1230	1170	1315	1160	1235	1240	1365	1360	1450	1480
3	M	900	950	800	950	920	1080	1160	1280	1320	1330	1395
4	M	1240	1300	1240	1365	1350	1570	1555	1580	1630	1685	1655
5	M	965	1030	990	1085	980	1100	1195	1210	1260	1330	1345
6	M	945	995	945	1045	960	1150	1325	1360	1390	1365	1410
7	F	1370	1450	1220	1390	985	1260	1440	1575	1660	1950	2200
8	F	1210	1320	1260	1340	1130	1230	1330	1515	1655	1890	2075
9	F	1425	1460	1380	1445	1410	D					
10	F	1230	1300	1210	1500	1415	1545	1770	1980	2010	2380	2560
11	F	1250	1270	1185	1340	1310	1370	1575	1760	1905	2205	2380
12	F	1235	1305	1200	1395	1340	1450	1555	1735	2005	2300	2395

TABLE I (c) All the pharyngeal nerves cut

No.	Sex	Body weight (in mg) on Day: 1 (B.R.)	1 (A.R.)	2	3	4	5	6	7	8	9	10
1	M	1050	1185	1070	1165	1030	1185	1110	1300	1260	1370	1420
2	M	1050	1190	1085	1240	1075	1285	1345	1440	1410	1570	1500
3	M	1040	1140	1055	1100	985	D					
4	M	985	1095	1005	1155	1040	1120	1245	1350	1340	1415	1380
5	M	1110	1225	1110	1255	1155	1300	1325	1435	1555	1540	1530
6	F	1360	1490	1325	1605	1420	1465	1630	1820	2050	2100	2590
7	F	1160	1270	1195	1385	1260	1305	1530	1615	1840	1830	2170
8	F	1280	1420	1350	1400	1395	1350	1630	1630	1835	1975	2205
9	F	1335	1420	1370	1330	1265	1190	D				
10	F	1240	1345	1260	1530	1340	1495	1815	1960	2230	2270	2695
11	F	1475	1630	1520	1650	1580	1665	1850	2010	2200	2380	2870
12	F	1240	1350	1270	1410	1260	1315	1635	1660	1855	1950	2110

TABLE I (d) Recurrent nerve cut

No.	Sex	Body weight (in mg) on Day: 1 (B.R.)	1 (A.R.)	2	3	4	5	6	7	8	9	10
1	M	1090	1145	1075	1180	1070	1120	1260	1220	1430	1365	1430
2	M	1030	1110	1050	1120	1100	1150	1270	1305	D		
3	M	1135	1255	1180	1225	1180	1190	1460	1470	D		
4	M	1080	1160	1100	1190	1030	1140	1235	1250	1335	1435	1400
5	M	965	1050	950	1055	980	1125	1040	1080	1260	1265	1165
6	M	1020	1140	1090	1170	1110	1140	1215	1185	1340	1260	1250
7	F	1405	1530	1470	1600	1490	1420	1590	1630	1770	1920	1955
8	F	1335	1400	1345	1410	1515	1615	1690	1745	2010	2045	2115
9	F	1120	1165	1130	1320	1260	1300	1290	1455	1490	1625	1710
10	F	1310	1415	1340	1310	1290	D					
11	F	1260	1360	1265	1325	1280	1390	1370	1475	1450	1610	1735
12	F	1250	1305	1250	1425	1360	1450	1490	1455	1650	1640	1610
13	F	1275	1360	1310	1450	1430	1520	1430	1355	1430	1550	1610
14	F	1140	1220	1150	1295	1180	1180	1390	D			

TABLE I (e) 2 Frontal connectives cut (male)

Response to Treatment	No.	Body weight (in mg) on Day: 1 (B.R.)	1 (A.R.)	2	3	4	5	6	7	8	9	10
Weight gain	1	1060	1100	1025	1130	1165	1190	1215	1300	1270	1330	1385
	2	1020	1045	960	1130	1020	1240	1160	1330	1415	1380	1365
	3	1060	1130	1060	1240	1090	1120	1250	1280	1395	1350	1360
Weight constant	4	1190	1275	1175	1235	1160	1110	1175	1175	1125	1140	1190
	5	1020	1110	1060	975	920	835	915	990	1035	1105	1075
	6	1100	1150	1070	1365	1040	1085	1100	1030	1140	1220	1240
	7	1020	1085	970	1070	980	1030	1030	1040	1150	1170	1205
	8	1110	1155	1050	1325	1230	1185	1215	1340	1210	1330	1380
	9	1025	1050	985	1130	995	1010	1040	1100	1145	1230	1300
	10	970	1000	945	1170	1000	1080	1055	1060	1035	1170	1170
	11	1015	1060	970	1175	1050	1090	1020	1050	1100	1130	1230
	12	960	990	955	1080	975	1035	1145	1150	1140	1120	1160
Dead before Day 10	13	1150	1220	1140	1255	1185	1225	1500	D			
	14	970	1030	970	1000	D						

TABLE I (e) 2 Frontal connectives cut (female)

Response to Treatment	No.	Body weight (in mg) on Day: 1 (B.R.)	1 (A.R.)	2	3	4	5	6	7	8	9	10
Weight gain	1	1280	1340	1280	1490	1310	1490	1640	1735	1700	1855	2010
	2	1275	1295	1200	1300	1310	1385	1475	1680	1760	1835	1920
	3	1185	1235	1180	1410	1200	1300	1370	1445	1590	1800	1835
	4	1260	1335	1235	1470	1320	1305	1330	1430	1560	1660	1685
Weight constant	5	1340	1395	1290	1340	1255	1215	1305	1340	1260	1375	1400
	6	1140	1185	1060	1365	1095	1120	1230	1235	1315	1320	1285
	7	1130	1180	1085	1170	1090	1170	1080	1135	1180	1210	1310
	8	1310	1390	1240	1230	1150	1180	1230	1280	1185	1180	1185
	9	1380	1445	1320	1365	1295	1340	1235	1340	1485	1380	1360
Dead before Day 10	10	1260	1340	1210	1415	1225	1335	D				
	11	1350	1425	1350	1235	1045	D					
	12	1270	1320	1265	1500	1260	D					
	13	1200	1230	1190	1205	1190	D					
	14	1115	1190	1105	1065	D						
	15	1305	1415	1280	1170	D						

TABLE I (f) Recurrent nerve + 2 Frontal connectives cut

Response to Treatment	No.	Sex	Body weight (in mg) on Day: 1 (B.R.)	1 (A.R.)	2	3	4	5	6	7	8	9	10
Weight gain	1	M	1040	1115	1070	1230	1080	1110	1160	1025	1195	1395	1325
	2	F	1240	1375	1310	1360	1335	1290	1430	1460	1775	1930	2065
	3	F	1405	1460	1385	1535	1420	1605	1375	1500	1560	1685	1925
	4	F	1255	1300	1255	1470	1300	1240	1360	1360	1305	1510	1705
	5	F	1265	1320	1270	1390	1340	1220	1380	1500	1575	1580	1860
Weight constant	6	M	985	1080	1030	1120	1000	1090	1010	935	1125	1060	1040
	7	F	1285	1375	1325	1450	1345	1270	1330	1220	1270	1240	1250
	8	F	1250	1345	1310	1350	1390	1455	1315	1280	1325	1335	1360

TABLE I (f) Recurrent nerve + 2 Frontal connectives cut

Response to Treatment	No.	Sex	Body weight (in mg) on Day: 1 (B.R.)	1 (A.R.)	2	3	4	5	6	7	8	9	10
Dead before Day 10	9	M	1110	1190	1090	1165	1055	930	1000	1035	1060	950	D
	10	F	1370	1460	1380	1420	1260	1315	1230	1170	1210	1325	D
	11	M	1160	1340	1270	1315	1185	1050	965	850	D		
	12	M	1010	1095	1030	1070	1020	1015	970	900	D		
	13	M	1040	1140	1050	1110	1010	955	870	D			
	14	M	1000	1045	995	1070	925	860	730	D			
	15	M	1010	1070	1025	1025	960	875	785	D			
	16	F	1170	1230	1190	1275	1250	1120	965	D			
	17	F	1235	1280	1230	1300	1160	1020	1010	D			
	18	M	1200	1315	1260	1270	1220	1005	D				
	19	M	925	980	955	1015	930	875	D				
	20	M	930	975	895	1085	930	830	D				
	21	F	1260	1325	1250	1210	955	1005	D				
	22	M	1130	1220	1150	1150	960	D					
	23	M	1050	1090	1035	1020	885	D					
	24	F	1225	1290	1225	1300	1240	D					
	25	F	1220	1290	1230	1185	1130	D					
	26	F	1350	1430	1360	1380	1320	D					
	27	M	1200	1340	1285	1320	D						
	28	F	1365	1430	1380	1430	D						

TABLE I (g) Removal of the frontal ganglion

Response to Treatment	No.	Sex	Body weight (in mg) on Day: 1 (B.R.)	1 (A.R.)	2	3	4	5	6	7	8	9	10
Weight gain	1	F	1300	1315	1155	1410	1265	1150	1420	1530	1415	1685	1735
Weight constant	2	M	1010	1030	980	1080	970	950	1005	1030	970	935	950
	3	M	1030	1060	985	1205	1150	1130	1160	1170	1105	1065	1075
	4	M	1105	1210	1060	1110	1090	995	1010	950	1040	1030	1065
	5	F	1280	1360	1180	1500	1310	1245	1220	1215	1180	1375	1450
	6	F	1245	1260	1120	1440	1285	1270	1420	1480	1360	1400	1520
	7	F	1250	1305	1150	1380	1225	1210	1110	1240	1225	1260	1320
Weight loss	8	M	1030	1105	1010	990	950	840	785	675	670	710	660
	9	M	930	975	855	920	940	825	760	785	820	740	680
	10	M	1120	1165	1000	1090	1020	1040	900	880	835	860	760
	11	F	1255	1310	1160	1315	1115	1060	1030	980	1040	1000	930
	12	F	1275	1295	1185	1510	1290	1085	930	1010	950	945	895

TABLE I (g) Removal of the frontal ganglion

Response to Treatment	No.	Sex	Body weight (in mg) on Day: 1 (B.R.)	1 (A.R.)	2	3	4	5	6	7	8	9	10
	13	M	970	1000	930	945	920	860	875	855	780	670	D
	14	F	1325	1360	1185	1115	970	960	1055	960	990	965	D
	15	F	1220	1270	1180	1255	910	860	810	860	890	920	D
	16	M	1115	1145	1015	1120	1030	950	960	905	770	D	
	17	M	975	1000	940	990	930	875	800	735	650	D	
	18	M	985	1000	870	1080	895	815	800	805	830	D	
	19	F	1200	1230	1190	1430	1240	1190	1140	1170	990	D	
Dead	20	M	1000	1015	860	1065	875	790	800	760	D		
before	21	F	1330	1350	1170	1435	1215	1150	940	870	D		
Day 10	22	M	1010	1060	900	880	840	860	710	D			
	23	M	1050	1090	940	1110	985	890	730	D			
	24	M	1045	1090	990	990	950	890	855	D			
	25	M	1060	1125	970	980	940	820	730	D			
	26	F	1275	1325	1245	1240	970	885	770	D			
	27	F	1340	1400	1310	1290	960	1105	1025	D			
	28	F	1225	1290	1190	1370	1140	1090	1040	D			
	29	M	1160	1215	1110	1200	905	765	D				

TABLE I (g) Removal of the frontal ganglion

Response to Treatment	No.	Sex	Body weight (in mg) on Day: 1 (B.R.)	1 (A.R.)	2	3	4	5	6	7	8	9	10
Dead before Day 10 (cont'd.)	30	M	1010	1040	890	1000	865	770	D				
	31	M	1085	1160	1090	1180	1080	880	D				
	32	M	1065	1100	1040	1035	1050	940	D				
	33	F	1340	1380	1290	1550	1465	1320	D				
	34	F	1240	1365	1185	1050	935	930	D				
	35	F	1330	1430	1310	1375	1120	950	D				
	36	F	1350	1440	1120	1210	1250	1050	D				
	37	M	1090	1140	995	1015	865	D					
	38	M	1030	1075	995	1010	810	D					
	39	M	1000	1080	950	1080	990	D					
	40	F	1235	1280	1125	1335	1220	D					
	41	F	1470	1545	1345	1455	1030	D					
	42	F	1430	1510	1415	1315	1180	D					
	43	F	1230	1285	1175	1395	1280	D					
	44	F	1200	1280	1150	1260	D						
	45	F	1345	1400	1285	1530	D						
	46	F	1245	1270	1115	1320	D						

TABLES II - IX

Daily body weight changes of fifth instar *Locusta* nymphs used in Experiments 2-9 of Section II (Chapter III).

TABLE II Experiment 2.

Group (a) Recurrent nerve cut.

No.	Weight (in mg) on Day: 1 (B.R.)	1 (A.R.)	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	567	651	620	803	800	820	1201	1265	1221	1185	D						
2	644	706	675	902	849	815	1060	1117	1199	1458	1560	1491	1620	1570	1466		
3	640	695	665	912	800	747	1010	1021	D								
4	600	657	637	868	839	844	930	968	1051	1053	D						
5	460	516	495	711	621	619	737	767	740	838	901	953	1068	1063	976		
6	531	571	550	747	717	681	940	937	995	1093	1211	1173	1130	1046			
7	453	510	495	678	716	626	845	910	998	1070	1153	1134	1101	1014			
8	578	641	614	746	808	801	903	929	D								
9	542	603	580	781	745	925	D										
10	600	663	637	895	807	744	965	975	940	1114	1253	1165	1324	1437	1455	1424	1325
11	621	660	629	884	843	804	941	D									
12	461	499	482	648	584	588	744	770	891	991	1155	1015	1043	978			
13	466	508	492	692	686	802	898	943	1011	1116	1189	1147	1119	954			
14	584	625	597	742	699	751	850	938	935	D							

TABLE II cont'd.

Group (b) Operated controls.

No.	Weight (in mg) on Day: 1 (B.R.)	1 (A.R.)	2	3	4	5	6	7	8	9	10	11	12	13
1	414	464	436	562	668	784	835	892	981	1031	1130	1122	1030	
2	494	532	493	677	787	842	982	1094	1216	1276	1385	1353	1248	
3	619	665	625	799	854	1023	1158	1344	1485	1615	1715	1670	1650	1486
4	432	474	440	605	715	760	890	951	1096	1168	1237	1227	1081	
5	624	682	660	838	880	991	1088	1211	1363	1432	1496	1615	1595	1447
6	536	585	545	642	853	912	1023	1168	1264	1343	1505	1469	1446	1319
7	451	509	465	583	704	795	864	921	983	1123	1213	1171	1054	
8	594	646	616	809	925	1069	1129	1259	1399	1535	1615	1575	1459	

TABLE III Experiment 3.

Group (a) 1 Frontal connective cut (proximal).

No.	Weight (in mg) on Day: 1 (B.R.)	1 (A.R.)	2	3	4	5	6	7	8	9	10	11	12	13
1	565	583	549	825	861	989	1094	1292	1422	1470	1443	1343		
2	500	530	500	730	859	857	1065	1198	1342	1466	1444	1420	1313	
3	507	531	489	755	845	953	1111	1154	1331	1438	1485	1467	1346	
4	543	558	520	843	892	992	1173	1339	1440	1455	1416	1326		
5	505	532	476	682	781	823	804	D						
6	510	532	499	668	790	911	1014	1160	1266	1403	1365	1346	1231	
7	531	545	519	772	894	926	1152	1311	1438	1545	1504	1403		
8	501	513	474	687	794	943	1010	1204	1324	1395	1375	1278		
9	436	460	428	628	759	818	919	1002	1153	1201	1212	1204	1112	
10	453	475	450	533	650	777	924	942	1062	1161	1224	1215	1095	
11	455	470	448	620	663	765	904	993	1116	1173	1195	1171	1147	1052
12	420	430	395	557	548	D								
13	465	478	454	665	729	805	889	972	1108	1175	1169	1154	1068	
14	447	477	445	662	697	873	959	1095	1190	1315	1297	1286	1192	
15	439	450	432	620	732	788	883	953	1085	1176	1234	1199	1169	1066
16	462	499	460	602	670	747	867	956	1091	1177	1238	1229	1121	

TABLE III cont'd.

Group (b) 1 Frontal connective cut (distal).

No.	1 (B.R.)	1 (A.R.)	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	Weight (in mg) on Day:															
1	553	662	586	710	752	817	943	1094	1103	1341	1467	1479	1438	1347		
2	529	593	519	616	673	711	868	959	1046	1246	1315	1384	1565	1535	1505	1387
3	591	683	573	698	766	829	997	1118	1156	1363	1488	1560	1540	1419		
4	525	585	537	739	846	755	917	951	1039	1193	1334	1420	1379	1357	1263	
5	525	621	583	561	D											
6	510	561	520	681	729	766	904	1002	982	1326	1494	1520	1515	1505	1389	
7	449	484	448	616	650	715	792	889	909	1136	1105	1106	1080	1071	978	
8	415	492	444	524	591	680	710	782	874	1023	1034	1022	955			
9	477	579	556	650	715	799	806	875	1050	1138	1165	1249	1311	1263	1239	1078
10	496	583	560	551	D											
11	426	470	449	528	536	645	712	845	983	1072	1169	1190	1175	1101		
12	499	614	566	660	703	750	855	889	1139	1290	1295	1203				
13	429	480	441	586	622	699	749	833	937	1043	1162	1131	1114	1047		
14	451	529	470	581	632	718	784	835	1009	1142	1246	1255	1236	1160		

TABLE III cont'd.

Group (c) Operated controls.

No.	1 (B.R.)	1 (A.R.)	2	3	4	5	6	7	8	9	10	11	12	13	14
	Weight (in mg) on Day:														
1	424	467	409	540	590	672	746	802	950	1099	1116	1110	1033		
2	485	514	478	631	693	771	868	943	1094	1223	1280	1256	1161		
3	473	519	456	610	757	863	989	1040	1103	1244	1287	1242	1151		
4	526	576	541	642	758	879	981	1086	1175	1334	1480	1565	1530	1505	1381
5	436	468	435	536	546	631	730	818	928	1070	1171	1235	1209	1104	
6	463	500	456	615	667	760	846	901	1034	1190	1135	1066			
7	494	518	484	698	745	851	983	1016	1079	1181	1225	1213	1150		
8	543	572	523	744	806	920	941	1167	1355	1422	1468	1429	1311		
9	584	619	589	801	849	965	1064	1093	1284	1442	1510	1525	1505	1391	
10	439	474	423	561	633	709	794	911	1067	1211	1235	1195	1123		
11	521	557	520	757	823	864	1043	1141	1289	1422	1375	1251			
12	544	602	551	766	938	933	1004	1108	1163	1287	1362	1565	1585	1545	1444
13	522	553	522	741	809	942	1067	1107	1183	1344	1395	1379	1281		
14	577	620	583	742	772	858	1018	1167	1342	1510	1595	1610	1570	1451	
15	429	472	425	574	602	606	789	858	986	1087	1165	1177	1146	1064	
16	555	580	538	616	631	D									
17	609	626	595	723	871	1029	1224	1235	1399	1570	1585	1570	1472		
18	466	507	474	504	D										

TABLE IV Experiment 4.

Group (a) 2 Frontal connectives cut (proximal).

No.	Weight (in mg) on Day: 1 (B.R.)	1 (A.R.)	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	470	498	464	563	565	531	502	513	589	D							
2	584	628	582	683	616	902	952	1034	1010	1111	1047	1040	1251	1295	1253	1227	1160
3	558	600	562	603	572	598	667	671	776	D							
4	470	499	472	469	515	558	569	570	567	582	D						
5	518	532	510	622	575	726	734	776	793	738	740	689	788	797	D		
6	484	501	466	471	503	D											
7	489	508	469	522	613	667	700	788	820	866	904	971	945	972	923	901	
8	519	572	534	634	602	582	671	677	684	D							
9	584	631	587	661	673	772	895	890	974	1070	D						
10	650	680	652	719	788	879	958	1016	1303	1158	1106	1368	1364	1433	1396	1367	1316
11	511	550	523	646	635	715	806	843	949	1003	1044	1168	1204	1191	1157	1138	
12	588	636	596	586	591	645	680	678	713	688	882	D					
13	474	528	489	473	475	479	539	638	588	546	523	537	499	D			
14	589	653	620	600	D												
15	557	575	553	531	587	608	D										
16	601	644	622	713	836	817	775	822	874	826	813	812	D				

TABLE IV cont'd.

Group (a) cont'd.

No.	Weight (in mg) on Day: 1 (B.R.)	1 (A.R.)	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
17	609	639	564	624	604	619	655	D									
18	534	557	496	D													
19	485	513	486	523	612	652	662	701	781	832	840	852	804	793	770	D	
20	475	512	465	622	660	629	638	645	683	650	751	748	768	706	D		
21	496	507	466	597	588	600	715	737	820	809	867	938	1041	1074	977	931	
22	516	557	533	561	D												
23	580	615	585	652	624	750	752	675	742	D							
24	549	592	560	700	798	915	973	1030	1149	1183	1141	1178	1137	1103	1081		
25	544	584	554	626	614	837	746	731	735	D							
26	493	523	501	581	643	645	671	679	660	733	629	677	627	609	601	D	
27	578	646	615	712	680	690	652	629	668	630	649	634	614	620	588	556	D
28	480	518	479	525	500	535	D										
29	466	482	463	535	584	630	626	674	738	697	663	627	585	553	D		
30	486	513	496	D													
31	483	525	501	539	582	610	542	498	516	547	559	616	670	656	670	D	

TABLE IV cont'd.

Group (a) cont'd.

No.	Weight (in mg) on Day: 1 (B.R.)	1 (A.R.)	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
32	483	504	455	523	587	562	624	605	740	914	899	871	990	1049	989	940	907
33	528	562	516	653	666	772	848	919	1007	892	842	1065	1002	1068	1027	1002	960
34	553	599	561	639	599	763	778	840	958	988	993	910	939	1245	1195	1158	1113
35	503	526	483	562	588	665	673	865	858	823	854	897	1006	1115	1162	1079	1007
36	540	565	517	563	542	591	562	595	554	667	626	575	693	721	653	714	762
37	590	641	611	701	763	860	888	956	1020	1041	1024	1087	1146	1162	1204	1169	1116
38	541	569	539	581	694	707	729	790	912	902	894	950	975	967	982	946	903
39	524	543	494	592	701	690	812	809	785	903	803	848	983	966	1011	1093	1080
40	525	573	528	555	550	656	640	707	649	621	714	658	692	712	726	772	702
41	648	680	649	699	739	808	881	926	929	1046	1058	1209	1219	1298	1280	1239	1167
42	511	533	498	635	726	657	761	816	775	783	831	770	806	861	882	880	845
43	475	537	508	537	590	686	765	808	765	783	862	830	945	1079	1057	1165	1154
44	533	558	533	584	613	607	738	646	684	676	602	643	727	717	677	625	604
45	556	612	563	704	678	679	813	926	870	815	746	795	917	952	976	922	905
46	574	630	577	632	576	693	754	714	730	707	701	755	708	673	633	681	690
47	486	514	493	557	610	643	723	819	817	748	732	834	855	865	969	907	883

TABLE IV cont'd.

Group (a) cont'd.

No.	Weight (in mg) on Day: 17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
32	871															
33	910	885														
34	1069															
35	952															
36	722	770	793	773	780	771	738	D								
37	1055															
38	857															
39	1146	1083	1041	993												
40	800	869	890	904	973	966	1011	1057	1039	1006	995	952	926	930	860	D
41	1103															
42	820	D														
43	1124	1011	923													
44	584	D														
45	864															
46	650	669	654	646	655	648	642	606	D							
47	823															

TABLE IV cont'd.

Group (b) Operated controls.

No.	1 (B.R.)	1 (A.R.)	2	3	4	5	6	7	8	9	10	11	12	13	14
						Weight (in mg) on Day:									
1	607	650	609	692	984	1090	1255	1359	1484	1685	1770	1665	1625	1490	
2	564	608	561	752	932	1067	1076	1078	D						
3	605	631	589	733	882	970	1106	1192	1335	1530	1655	1625	1585	1376	
4	595	637	606	842	970	1104	1198	1306	1443	1585	1590	1560	1520	1471	D
5	499	537	513	714	819	940	1044	1100	1261	1343	1366	1343	1245		
6	634	673	631	768	909	919	887	D							
7	498	529	488	679	831	878	994	1094	1215	1344	1382	1341	1232		
8	611	651	608	744	1035	1040	1257	1403	1565	1675	1630	1600	1565	1440	
9	491	527	502	651	801	915	965	1087	1175	1250	1405	1363	1265		
10	484	520	487	646	770	876	954	1091	1189	1268	1360	1312	1282	1138	
11	622	665	627	845	1019	1118	1179	1325	1466	1625	1750	1740	1705	1570	
12	496	532	500	710	748	829	962	1029	1170	1247	1275	1246	1150		
13	498	547	513	674	810	853	977	1060	1233	1321	1403	1363	1324	1236	
14	595	643	614	833	1004	1163	1261	1396	1575	1695	1705	1655	1540		
15	462	494	465	672	770	855	1001	1089	1218	1288	1255	1180			
16	544	573	535	758	843	1011	1058	1140	1320	1483	1552	1550	1505	1378	
17	601	627	586	774	939	970	1135	1286	1461	1605	1640	1740	1735	1690	D
18	483	512	488	653	836	923	1044	1078	1179	1274	1291	1267	1138		
19	460	490	458	568	805	875	969	1077	1159	1251	1292	1262	1168		
20	477	500	474	645	731	833	938	1024	1192	1322	1329	1307	1198		

TABLE V Experiment 5.

Group (a) 2 Frontal connectives cut (distal).

No.	Weight (in mg) on Day: 1 (B.R.)	1 (A.R.)	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	447	560	521	569	565	624	758	799	728	883	847	871	915	902	867	D	
2	513	589	530	575	631	684	759	794	881	871	1002	991	1009	1016	1055	1019	1029
3	534	620	545	567	D												
4	542	664	620	684	776	802	926	1019	1031	1142	1267	1405	1410	1411	1377	1228	
5	581	647	616	708	768	961	945	958	1085	1233	1332	1505	1510	1535	1496	1334	
6	442	563	525	505	D												
7	501	615	566	860	633	701	732	846	891	894	860	930	865	D			
8	445	527	495	531	587	607	706	761	757	924	953	981	982	935	886		
9	429	538	507	510	511	D											
10	555	687	651	609	760	811	749	1001	979	871	969	1000	1209	1053	1034	984	913
11	479	584	546	559	575	573	648	686	756	832	878	945	926	895	850	812	
12	444	556	499	494	D												
13	438	506	444	453	471	486	477	518	461	492	463	478	524	539	526	495	D
14	535	623	589	571	556	D											
15	564	621	575	694	714	747	834	882	970	1090	1208	1316	1401	1400	1368	1256	
16	553	627	575	570	646	663	634	606	624	599	582	568	D				

TABLE V cont'd.

Group (a) cont'd.

No.	Weight (in mg) on Day: 1 (B.R.)	1 (A.R.)	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
17	500	552	516	595	641	713	693	707	718	804	837	808	779	755	760	886	875
18	588	639	591	695	742	791	857	891	1097	1240	1267	1239	1180	1127			
19	424	502	471	456	D												
20	423	531	502	483	472	D											
21	469	574	528	557	D												
22	511	646	610	680	696	660	605	709	652	666	835	840	790	773	928	905	993
23	420	508	485	471	D												
24	496	574	528	578	589	605	637	657	696	758	760	799	806	759	740	784	775

No.	17	18	19	20
2	1017	1005	D	
17	862	D		
22	950	933	893	D
24	794	D		

TABLE V cont'd.

Group (b) Operated controls.

No.	Weight (in mg) on Day: 1 (B.R.)	1 (A.R.)	2	3	4	5	6	7	8	9	10	11	12	13	14
1	552	587	552	801	880	911	1130	1138	1336	1515	1650	1635	1605	1477	
2	462	499	474	569	640	D									
3	497	521	494	704	711	818	874	960	1074	1116	1174	D			
4	423	456	429	613	619	722	668	D							
5	587	627	590	806	852	970	1069	1233	1440	1515	1685	1695	1655	1525	
6	472	501	469	578	621	700	852	865	1033	1156	1170	1153	1076		
7	429	453	420	582	654	686	797	902	1005	1093	1228	1198	1112		
8	531	565	531	680	797	858	1087	1196	1358	1525	1505	1482	1369		
9	572	642	593	562	D										
10	627	650	621	833	827	1031	1177	1320	1338	1565	1620	1670	1655	1605	1505
11	474	498	460	675	678	710	831	1047	1121	1191	1192	1159	1077		
12	591	612	582	736	816	908	1007	1093	1211	1362	1555	1525	1400		

TABLE VI Experiment 6.

Group (a) Pharyngeal nerves cut.

No.	Weight (in mg) on Day: 1 (B.R.)	1 (A.R.)	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	546	636	570	680	595	805	839	970	1019	1192	1321	1435	1428	1387	1355		
2	542	619	538	589	626	761	778	925	1098	1222	1178	1395	1426	1485	1429	1385	
3	558	675	603	746	911	972	1092	1178	1372	1459	1555	1446	1366	1324			
4	481	533	491	617	609	778	817	872	1004	1068	1134	1189	1262	1282	1241	1217	1192
5	562	606	556	658	617	810	782	992	1055	1141	1299	1382	1450	1437	1357	1318	1243
6	511	553	503	526	518	D											
7	489	596	545	619	655	696	714	758	958	1008	1108	1202	1233	1193	1162		
8	479	540	496	584	620	660	710	800	929	900	1007	1153	1152	1112	1083		
9	547	615	582	557	707	826	904	1028	1037	1195	1368	1436	1500	1461	1411	1370	
10	457	523	471	515	482	619	642	742	917	950	1100	1138	1188	1219	1190	1161	
11	594	637	606	600	651	739	780	884	998	1120	1184	1202	1162	1115			
12	536	594	548	612	570	739	808	948	1035	1131	1228	1401	1479	1525	1476	1436	1397
13	577	642	593	561	566	D											
14	611	692	627	607	606	683	779	936	1006	1054	1229	1308	1432	1456	1386	1342	
15	584	635	587	695	678	783	852	934	1076	1104	1162	1407	1500	1555	1515	1470	
16	473	547	490	576	635	721	754	855	944	1066	1151	1238	1218	1177	1116		
17	539	630	576	685	673	800	913	1015	988	1103	1078	1015	1084	1228	1164	1104	1069
18	416	506	462	442	D												
19	461	536	480	595	603	640	765	862	975	1007	1122	1147	1118	1065	1016		

TABLE VI cont'd.

Group (a) cont'd.

No.	Weight (in mg) on Day: 1 (B.R.)	1 (A.R.)	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
20	498	583	524	556	542	703	771	848	939	948	1087	1193	1224	1177	1148		
21	453	537	488	524	485	625	692	746	875	937	990	1043	1094	1110	1064	1074	1031
22	569	685	613	714	794	922	968	1048	1067	1195	1302	1360	1411	1501	1555	1550	1500
23	599	663	604	695	761	952	979	1071	1099	1391	1489	1590	1595	1555	1435		
24	512	566	529	548	532	613	764	846	1039	1030	1033	1140	1251	1252	1232	1338	1374
25	464	544	511	613	669	718	799	850	939	1007	1093	1221	1235	1233	1141	1109	1064
26	489	546	508	573	635	777	738	939	1064	1101	985	928	886	829			
27	524	610	575	668	727	780	914	943	968	1061	1187	1258	1375	1449	1441	1384	1298
28	514	582	559	542	571	582	D										
29	410	476	531	587	518	677	693	787	973	1000	1133	1158	1139	1110	974		
30	488	539	505	509	624	694	760	797	810	893	992	1017	1070	1000	1020	988	1027
31	555	649	572	596	503	678	670	817	1001	1053	1065	1074	1162	1229	1169	1244	1205
32	596	685	634	769	656	866	868	919	1048	1185	1240	1325	1431	1432	1392	1535	1488
33	559	666	602	761	844	897	1013	1084	1145	1153	1220	1161	1126	1085	1007	992	1017
34	432	484	441	449	470	608	590	749	777	811	849	982	1129	1185	1136	1097	
35	539	636	582	626	584	792	873	933	1039	1128	1147	1200	1245	1239	1186	1304	1345
36	535	603	555	643	671	762	846	948	980	1088	1188	1265	1365	1438	1535	1500	1447
37	457	507	480	595	650	820	822	881	1020	1063	1146	1206	1256	1228	1201		
38	500	538	467	501	574	633	691	749	805	868	891	888	914	925	896	915	960

TABLE VI cont'd.

Group (a) cont'd.

No.	Wt. (in mg) on Day: 17	18	19	20
21	988	D		
24	1396	1355	1328	1271
25	D			
27	1242			
30	1044	D		
31	1181			
32	1461	1409		
33	D			
35	1351	1294	1232	D
36	1352			
38	957	D		

TABLE VI cont'd.

Group (b) Operated controls

No.	Weight (in mg) on Day: 1 (B.R.)	1 (A.R.)	2	3	4	5	6	7	8	9	10	11	12	13	14
1	627	652	631	828	865	972	1061	1223	1479	1545	1655	1630	1615	1430	
2	539	567	522	654	833	860	935	987	D						
3	511	542	509	648	734	856	956	1111	1193	1345	1402	1398	1366	1251	
4	629	664	618	814	875	975	1126	1266	1454	1633	1648	1598	1446		
5	528	554	516	565	702	797	825	889	1000	1036	1044	1142	1128	1093	D
6	501	540	485	619	680	796	903	1105	1239	1342	1461	1445	1418	1309	
7	502	542	501	626	670	774	852	1020	1164	1216	1360	1403	1390	1362	1248
8	441	485	433	568	641	688	890	1019	1103	1171	1206	1180	1086		
9	460	492	471	566	D										
10	438	494	448	570	615	702	815	979	1072	1169	1249	1213	1123		
11	428	474	432	597	620	689	879	969	1063	1159	1251	1225	1125		
12	430	470	418	554	556	665	815	952	985	1058	1169	1151	1062		
13	427	452	420	486	558	671	788	909	986	1050	1129	1124	1104	1006	
14	440	471	430	574	645	719	884	958	1084	1155	1175	1155	1054		
15	405	443	400	542	596	728	831	932	1024	1079	1168	1159	1069		

TABLE VII Experiment 7.

Group (a) Anterior pharyngeal nerves cut.

No.	Weight (in mg) on Day: 1 (B.R.)	1 (A.R.)	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	542	599	546	714	743	1022	1095	1194	1360	1535	1560	1540	1483	1387			
2	567	634	580	781	798	1015	1178	1311	1469	1615	1605	1565	1535				
3	526	617	568	671	680	796	947	994	1019	1211	1284	1357	1433	1505	1505	1483	D
4	527	585	544	799	788	873	1049	1192	1173	1435	1525	1575	1545	1427			
5	561	639	605	876	893	972	1157	1257	1369	1445	1545	1600	1570	1540	1505		
6	553	623	583	795	810	1010	1155	1273	1467	1510	1493	1470	1359				
7	558	640	605	766	857	1023	1178	1307	1489	1590	1555	1535	1438				
8	571	634	604	802	840	1025	1217	1365	1457	1465	1535	1500	1375				
9	444	487	465	666	743	795	923	1020	1143	1251	1271	1243	1158				
10	632	724	697	695	755	923	983	1079	1249	1402	1412	1645	1635	1715	1690	1670	
11	574	642	613	D													
12	468	563	533	669	681	749	837	912	1002	1112	1170	1231	1206	1188			
13	475	547	524	722	764	812	971	1072	1208	1346	1362	1319	1229				
14	441	519	503	657	712	792	847	974	1110	1217	1194	1172	1106				
15	463	523	494	620	656	722	796	924	974	1057	1116	1147	1216	1175	1161		
16	564	692	668	811	870	975	1106	1248	1382	1565	1655	1595	1555				
17	605	678	651	832	866	979	1069	1206	1378	1397	1510	1448	1428	1332			
18	474	567	535	587	D												

TABLE VII cont'd.

Group (b) Posterior pharyngeal nerves cut.

No.	1 (B.R.)	1 (A.R.)	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	Weight (in mg) on Day:																
1	522	610	548	718	783	845	955	1020	1141	1267	1455	1488	1470	1432	1326		
2	525	597	560	711	791	839	935	1048	1160	1277	1407	1360	1335	1227			
3	562	645	611	740	842	914	940	985	1109	1132	D						
4	499	584	551	610	737	796	800	867	991	1115	1133	1270	1358	1357	1329	1280	1246
5	425	470	426	456	578	665	658	737	851	916	1001	1040	1101	1199	1202	1159	1113
6	454	531	492	526	596	619	677	773	862	920	1038	1164	1168	1136	1107	1004	
7	534	645	581	574	D												
8	633	694	625	745	780	913	997	1041	1122	1285	1368	1447	1439	1415	1290		
9	514	566	512	503	D												
10	485	551	519	541	D												
11	424	539	504	600	D												
12	518	564	532	741	D												
13	458	504	467	598	711	742	873	929	990	1085	1061	1040	944				
14	439	482	426	662	767	848	930	992	1083	1174	1235	1178	1102				
15	559	648	605	953	997	1015	1137	1269	1246	1374	1535	1550	1550	1560	1535	1515	1401
16	521	628	604	731	787	873	896	1132	1177	1308	1500	1500	1510	1483	1402		

TABLE VII cont'd.

Group (c) Operated controls.

No.	Weight (in mg) on Day													
	1 (B.R.)	1 (A.R.)	2	3	4	5	6	7	8	9	10	11	12	13	14
1	410	448	410	497	545	681	741	881	956	1033	1149	1155	1124	1044	
2	584	609	595	623	629	D									
3	428	453	416	534	646	744	860	869	1061	1168	1186	1151	1068		
4	472	507	471	621	695	751	869	972	1128	1246	1320	1339	1320	1228	
5	444	475	440	555	622	756	806	834	875	1032	1144	1199	1179	1163	1065
6	450	481	446	613	698	800	886	1041	1192	1289	1304	1292	1206		
7	614	651	597	798	919	1032	1220	1352	1520	1615	1655	1655	1540		
8	603	646	613	772	870	1018	1179	1292	1388	1520	1615	1570	1450		
9	546	588	558	758	837	896	1035	1222	1445	1525	1595	1560	1465		
10	536	568	526	690	821	866	948	1066	1240	1389	1486	1463	1441	1310	

TABLE VIII Experiment 8.

Group (a) Frontal ganglion removed.

No.	Weight (in mg) on Day: 1 (B.R.)	1 (A.R.)	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	586	616	589	741	702	724	776	806	823	789	763	802	740	760	702	713	683
2	500	541	520	551	510	502	555	510	D								
3	540	566	538	582	603	576	576	632	627	815	805	929	825	888	856	840	896
4	630	666	639	710	652	637	708	835	767	740	782	783	757	731	793	780	772
5	594	620	590	659	671	638	626	689	653	788	755	840	781	823	783	765	803
6	531	577	557	642	615	564	507	592	607	740	747	779	720	713	726	641	587
7	510	544	514	606	726	666	667	885	789	870	816	903	863	911	874	927	889
8	559	599	576	685	695	708	636	762	756	841	823	866	782	878	1016	924	884
9	438	464	438	557	545	476	464	503	459	468	430	D					
10	436	468	446	503	483	450	440	474	458	426	D						
11	471	499	480	527	573	540	481	417	D								
12	445	490	459	488	497	464	489	557	533	539	486	D					
13	441	476	440	595	597	552	589	592	603	679	690	664	615	556	550	D	
14	430	456	417	511	553	491	505	564	526	667	696	710	691	667	621	598	D
15	453	486	462	586	525	528	632	689	737	762	799	842	876	855	987	907	853
16	592	612	580	704	613	615	652	624	673	679	689	773	765	703	616	605	D
17	464	494	461	551	574	565	588	560	534	505	481	443	D				
18	435	459	422	487	456	470	453	451	462	460	469	414	D				
19	556	601	577	636	599	592	549	564	570	543	528	D					

TABLE VIII cont'd.

Group (a) cont'd.

No.	Weight (in mg) on Day: 17	18	19	20	21	22	23	24	25	26
1	D									
3	943	1102	1029	972	979	986	950	903	839	762
4	775	819	783	761	794	738	D			
5	804	782	733	D						
6	D									
7	821									
8	D									
15	692									

TABLE VIII cont'd.

Group (b) Operated controls.

No.	Weight (in mg) on Day: 1 (B.R.)	1 (A.R.)	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	527	546	514	658	773	812	1073	1110	1328	1416	1500	1438	1316			
2	596	624	601	765	846	939	1164	1323	1464	1645	1615	1550	1414			
3	539	580	545	674	713	784	855	948	1063	1147	1265	1421	1470	1444	1419	1310
4	582	614	578	635	693	829	867	981	1128	1082	1218	1384	1465	1449	1326	
5	473	513	474	564	644	705	802	974	963	1163	1190	1281	1268	1174		
6	439	466	429	677	686	737	799	938	1044	1146	1177	1235	1196	1112		
7	510	536	491	782	817	860	1076	1150	1294	1499	1510	1477	1362			
8	459	488	451	489	589	708	871	916	1097	1207	1228	1193	1101			

TABLE IX Experiment 9.

Group (a) 1 Labral nerve cut.

No.	Weight (in mg) on Day: 1 (B.R.)	1 (A.R.)	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	597	680	646	828	916	944	1058	1241	1381	1560	1660	1625	1605	1453		
2	520	655	619	692	751	819	1026	1056	1261	1419	1500	1515	1499	1358		
3	558	624	585	712	865	907	1027	1136	1325	1454	1510	1485	1391			
4	548	573	551	737	779	854	952	984	1194	1266	1437	1440	1505	1474	1447	1333
5	512	579	560	654	868	927	965	1083	1246	1353	1505	1635	1545	1505	1395	
6	416	495	463	519	640	758	805	937	1030	1109	1223	1183	1161			
7	419	501	456	546	670	701	800	901	1050	1128	1140	1135	1048			
8	489	545	524	587	668	694	801	994	1016	1129	1187	1148	1127			
9	427	494	469	600	726	784	850	940	1110	1187	1230	1208	1043			
10	434	501	482	522	640	723	773	890	1053	1100	1192	1158	1134			

TABLE IX cont'd.

Group (b) 2 Labral nerves cut.

No.	Weight (in mg) on Day: 1 (B.R.)	1 (A.R.)	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	575	660	600	660	734	806	794	900	982	1001	1064	1170	1175	1259	1300	1219	1151
2	509	654	593	546	526	505	499	485	476	443	D						
3	553	676	641	615	D												
4	520	634	601	577	571	D											
5	559	687	655	623	D												
6	590	683	619	574	557	549	D										
7	436	477	424	437	483	548	606	690	722	795	*907	1016	1036	1018	977	903	
8	480	585	526	539	663	648	715	*742	*853	*841	*957	1094	1159	1133	1085	1052	961
9	457	519	499	492	511	608	740	786	835	858	992	1165	1208	1191	1152	1131	1024
10	500	604	559	506	558	669	682	800	810	792	888	984	1067	1107	1170	1129	1082
11	438	543	521	482	D												
12	453	591	563	525	D												
13	447	559	529	479	477	501	604	653	677	764	711	*702	632	*643	*663	723	829
14	537	643	590	655	734	789	786	844	903	973	992	1031	1014	1023	1012	D	
15	560	665	588	570	D												
16	524	583	546	533	D												
17	603	709	641	619	592	D											
18	549	606	568	585	576	571	693	762	824	912	1041	1125	1284	1340	1488	1360	1237

TABLE IX cont'd.

Group (b) cont'd.

No.	Weight (in mg) on Day: 1 (B.R.)	1 (A.R.)	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
19	570	659	587	564	D												
20	517	644	558	544	535	510	491	474	551	668	658	741	796	793	810	772	747
21	450	533	502	486	475	457	453	502	472	488	476	D					
22	448	539	497	500	568	674	760	*824	831	955	1030	1125	1168	1202	1183	1136	960
23	516	553	514	584	699	732	822	*875	850	956	1065	1072	1137	1142	1102	1058	D
24	481	544	487	469	455	445	D										

No.	Weight (in mg) on Day: 17	18	19	20	21	22	23	24
1	1108	D						
10	996							
13	787	851	857	846	817	782	*747	D
20	D							

TABLE IX cont'd.

Group (c) Operated controls.

No.	Weight (in mg) on Day: 1 (B.R.)	1 (A.R.)	2	3	4	5	6	7	8	9	10	11	12	13	14
1	428	460	427	584	621	769	873	995	1116	1203	1220	1189	1111		
2	490	535	499	658	756	839	961	1100	1172	1269	1364	1332	1237		
3	588	616	576	764	888	937	992	1116	1223	1300	1459	1590	1635	1625	1485
4	469	497	463	591	699	806	844	1010	1109	1228	1295	1281	1262	1141	
5	449	487	453	619	677	828	899	991	1136	1186	1208	1168	1062		
6	596	616	592	737	839	1007	1086	1185	1130	1171	1322	1491	1550	1520	1382
7	417	456	414	555	667	769	821	942	1033	1169	1133	1026			
8	565	592	561	787	850	981	1096	1278	1469	1570	1625	1570	1470		
9	524	564	533	700	711	875	944	1144	1296	1364	1438	1415	1332		
10	612	643	614	762	992	1114	1186	1226	1397	1525	1605	1700	1660	1471	

TABLES X, XI

Daily body weight changes and faeces production in fourth and fifth instar *Locusta* nymphs used in Section III (Chapter III).

TABLE X Frontal ganglionectomy and faeces production.

Daily weight changes in fifth instars.

Operated controls.

No.	Sex	Body weight (in mg) on Day: 1	2	3	4	5	6	7	8	9	10	11	12	13
1	F	582	537	755	846	854	1034	1202	1310	1472	1575	1595	1555	1437
2	F	525	488	678	733	811	1020	1093	1239	1383	1472	1512	1474	1360
3	F	598	569	763	876	955	1105	1130	1470	1665	1735	1670	1555	
4	F	534	512	655	665	772	892	1000	1145	1315	1442	1515	1479	1349
5	F	561	540	722	814	943	1063	1138	1296	1458	1675	1685	1655	1535
6	F	595	578	777	846	942	1175	1245	1493	1635	1715	1685	1655	1520
7	F	552	530	801	880	911	1130	1138	1336	1515	1650	1635	1605	1477
8	F	587	556	806	852	970	1069	1233	1440	1515	1685	1695	1655	1525
9	F	531	511	680	797	858	1087	1196	1358	1515	1505	1482	1369	
10	M	459	431	624	685	731	830	935	1034	1137	1180	1168	1073	
11	M	437	409	562	620	695	811	907	1023	1153	1250	1244	1219	1061
12	M	498	472	607	668	731	878	910	1028	1127	1224	1241	1214	1122
13	M	447	425	635	689	782	903	958	1101	1183	1250	1222	1125	
14	M	495	477	725	786	804	966	1000	1125	1270	1335	1313	1197	
15	M	430	403	546	614	732	829	907	941	924	D			
16	M	446	420	505	516	506	D							

TABLE X Frontal ganglionectomy and faeces production.

Daily weight changes in fifth instars.

Operated animals: (a) Growth (over 100% increase in weight) and attempted moulting.

No.	Sex	Body weight (in mg) on Day: 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	F	541	468	634	670	633	637	706	750	804	823	890	877	860	871	1000	1075	1159	1249
2	F	628	581	787	765	752	744	754	880	1031	1001	1072	1117	1116	1131	1315	1400	1315	1248
3	F	574	531	606	673	648	592	797	875	965	914	1116	1154	1037	1100	1133	1089	1029	938
4	F	510	478	568	658	774	756	789	889	939	916	1002	1125	1041	1026	1062	1144	1081	1032
5	F	565	517	662	734	760	748	823	874	967	1009	1191	1236	1207	1170	1183	1147	1066	
6	M	498	470	593	661	763	773	828	687	757	933	1049	1050	1097	1136	1116	1049	958	
7	M	433	409	516	586	620	606	624	692	774	771	918	991	922	1000	1118	1061	988	
8	M	491	466	568	698	750	618	706	805	859	779	912	1116	1064	1060	1108	1051	978	
9	M	496	468	591	640	727	714	700	827	874	910	1102	1177	1092	1022	924	844		
10	M	467	434	502	568	675	675	670	773	823	869	1017	1063	1055	1006	930	832		
11	M	459	425	473	537	613	676	608	715	806	870	923	972	912	853	780	734		
12	F	557	481	723	666	779	801	907	938	1055	1040	1198	1143	1113	1031	897			
13	M	471	406	407	561	589	622	677	669	830	886	968	964	915	844				

No.	Sex	Wt. (in mg) on Day: 19	20	21	22
1	F	1253	1171	1086	943

TABLE X Frontal ganglionectomy and faeces production.

Daily weight changes in fifth instars.

Operated animals: (b) Growth (over 100% increase in weight) and no attempted moulting.

No.	Sex	Body weight (in mg) on Day: 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
14	F	568	530	677	636	731	742	826	874	912	1036	1078	990	1050	1119	1148	1094	1096	985
15	F	594	562	697	723	698	729	852	896	1041	1031	1109	1204	1197	1148	1198	1232	D	

No.	Sex	Body wt. (in mg) on Day: 19	20	21	22	23
14	F	950	987	944	950	D

TABLE X Frontal ganglionectomy and faeces production.

Daily weight changes in fifth instars.

Operated animals: (c) Reduced growth (50-100% increase in weight) and no attempted moulting.

No.	Sex	Body weight (in mg) on Day: 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
16	F	581	526	640	629	721	734	733	788	820	861	975	976	925	890	998	1031	1010	1026	D
17	M	464	395	509	503	574	583	585	631	652	685	779	778	736	708	796	821	809	820	D
18	F	566	504	640	637	715	818	813	810	827	814	852	868	831	839	826	770	678	609	D
19	M	475	451	529	559	562	603	653	696	787	746	838	881	842	781	835	800	D		
20	F	537	503	630	580	667	751	859	833	940	906	892	931	882	940	856	864	D		
21	F	589	531	627	640	640	668	689	805	850	816	970	1016	977	990	999	987	D		
22	M	483	439	532	598	678	616	579	560	578	650	719	719	746	724	689	643	D		
23	M	499	475	623	675	692	689	666	688	837	888	905	842	806	770	D				

TABLE X Frontal ganglionectomy and faeces production.

Daily weight changes in fifth instars.

Operated animals: (d) Little or no growth (0-50% increase in weight) and no attempted moulting.

No.	Sex	Body weight (in mg) on Day: 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
24	M	469	430	499	513	512	523	527	556	577	562	571	668	580	563	594	577	569	D
25	F	555	506	637	566	605	619	715	659	782	722	736	715	694	758	799	738	683	D
26	F	518	470	590	536	617	615	585	625	691	638	644	672	633	577	536	551	D	
27	F	622	572	691	682	642	601	572	533	529	546	552	546	585	500	D			
28	F	570	513	581	669	674	608	644	645	685	703	743	707	688	D				
29	F	556	484	597	545	615	647	600	641	611	579	588	586	561	D				
30	M	435	389	464	445	470	518	486	530	543	503	472	436	D					

TABLE X Frontal ganglionectomy and faeces production.

Daily faeces production in fifth instars.

Operated controls.

No.	Sex	Dry wt. of faeces (in mg) on Day: 3	4	5	6	7	8	9	10	11	Max. body weight gain (%)	Total weight faeces (mg)	Mean daily faeces production (mg/day)
1	F	43	38	62	86	152	150	80	78	41	174	730	81
2	F	33	42	42	80	145	148	85	62	52	188	689	77
3	F	39	66	79	118	172	168	106	48	13	190	809	90
4	F	36	41	50	79	135	117	77	101	46	184	682	76
5	F	46	39	60	116	125	116	89	93	45	200	729	81
6	F	42	60	57	113	155	183	86	57	15	188	768	85
7	F	54	57	57	103	179	155	86	75	37	199	803	89
8	F	51	48	60	139	180	158	93	96	67	189	892	99
9	F	36	56	70	102	145	154	113	51	16	185	743	83
Mean	F	42	50	60	104	154	150	91	73	37	189	761	85
10	M	32	43	41	80	101	97	55	48	22	157	519	58
11	M	22	38	55	83	100	120	71	57	25	186	571	63
12	M	38	60	51	93	113	93	50	39	18	149	555	62
13	M	35	45	48	75	110	96	54	48	4	180	515	57
14	M	38	60	40	90	114	112	44	59	18	170	585	65
Mean	M	33	49	49	84	108	104	55	50	17	168	549	61

For control and operated groups, operation body weight = 0%.

TABLE X Frontal ganglionectomy and faeces production.

Daily faeces production in fifth instars.

Operated animals: (a) Growth (over 100% increase in weight) and attempted moulting.

No.	Sex	Dry weight of faeces (in mg) on Day: 3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	Max. body weight gain (%)	Total weight faeces (mg)	Mean daily faeces production (mg/day)
1	F	26	21	37	19	33	43	54	31	36	72	32	60	63	88	75	118	29	32	8	-	132	877	46
2	F	48	58	24	21	39	66	94	73	93	156	131	159	120	66	33	-					123	1181	79
3	F	10	29	15	13	51	59	107	62	85	79	36	29	22	4	0	-					101	601	40
4	F	24	34	39	26	60	67	62	35	54	59	32	19	31	31	11	-					124	584	39
Mean	F	27	40	26	20	50	64	88	57	77	98	66	69	58	34	15	-					116	789	53
5	F	33	31	51	33	55	60	74	75	94	87	40	31	41	4	-						119	709	51
6	M	38	49	61	39	73	15	27	65	102	106	72	37	52	2	-						128	738	53
7	M	28	30	29	20	54	49	72	43	99	76	41	40	81	15	-						158	679	48
8	M	33	70	59	21	45	71	109	32	43	90	50	37	55	8	-						127	723	52
Mean	M	33	50	50	27	57	45	69	47	81	91	54	38	63	8	-						138	713	51
9	M	31	43	48	17	34	57	76	40	84	73	22	7	0	-							137	543	42
10	M	32	40	42	30	38	69	71	79	95	63	33	12	7	-							128	611	47
11	M	10	32	32	40	39	55	65	82	86	26	27	6	0	-							112	500	38
Mean	M	24	42	41	29	37	60	71	67	88	54	27	8	2	-							126	551	42
12	F	59	39	45	53	50	54	62	53	134	56	22	15	-								115	642	54
13	M	13	55	47	56	84	81	115	65	68	45	4	-									106	633	58

TABLE X Frontal ganglionectomy and faeces production.

Daily faeces production in fifth instars.

Operated animals: (b) Growth (over 100% increase in weight) and no attempted moulting.

No.	Sex	Dry weight of faeces (in mg) on Day: 3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	Max. body weight gain (%)	Total weight faeces (mg)	Mean daily faeces production (mg/day)
14	F	36	16	40	19	23	36	41	36	63	38	41	44	70	31	50	30	46	16	24	0	D	102	700	35
15	F	23	34	9	23	51	53	72	39	44	64	44	34	65	34	D							103	589	42
Mean	F	30	25	25	21	37	45	57	38	54	51	43	39	68	33	50	30	46	16	24	0	D	103	645	39

TABLE X Frontal ganglionectomy and faeces production.

Daily faeces production in fifth instars.

Operated animals: (c) Reduced growth (50-100% increase in weight) and no attempted moulting.

No.	Sex	Dry weight of faeces (in mg) on Day: 3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	Max. body weight gain (%)	Total weight faeces (mg)	Mean daily faeces production (mg/day)
16	F	46	29	42	33	30	39	50	38	46	35	24	22	33	18	6	0	D	77	491	31
17	M	51	59	57	27	19	25	32	24	43	49	36	23	32	5	3	0	D	59	485	30
18	F	33	27	39	30	34	30	38	33	35	33	42	32	58	21	32	5	D	53	522	33
19	M	24	31	29	20	30	38	57	25	44	43	9	20	29	6	D			85	393	28
20	F	30	21	42	38	49	20	55	30	22	34	24	46	30	8	D			75	449	32
21	F	34	26	25	35	39	39	51	29	30	45	34	24	36	9	D			73	456	33
22	M	37	65	51	23	29	13	27	49	26	38	29	43	26	3	D			54	459	33
23	M	31	52	46	40	47	38	52	42	25	3	0	5	D					81	381	32
Mean	M	36	52	46	28	31	29	42	35	35	33	19	23	29	5	3	0		70	430	31
Mean	F	36	26	37	34	38	32	49	33	33	37	31	31	39	14	19	3		70	480	32

TABLE X Frontal ganglionectomy and faeces production.

Daily faeces production in fifth instars

Operated animals: (d) Little or no growth (0-50% increase in weight) and no attempted moulting.

No.	Sex	Dry weight of faeces (in mg) on Day: 3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Max. body weight gain (%)	Total weight faeces (mg)	Mean daily faeces production (mg/day)
24	M	8	6	0	26	30	15	25	12	19	39	20	18	24	10	0	D	42	252	17
25	F	36	11	25	27	36	25	36	25	39	25	26	28	37	14	28	D	44	418	28
26	F	49	12	30	23	13	27	33	20	34	29	14	18	22	10	D		33	334	24
27	F	34	34	2	7	0	13	12	15	14	11	14	16	D				11	172	14
28	F	17	33	23	19	29	28	37	22	14	0	0	D					30	222	20
29	F	43	15	35	26	19	27	15	11	21	11	14	D					16	237	22
30	M	20	13	15	32	17	22	23	14	11	1	D						25	168	17
Mean	M	14	10	8	29	24	19	24	13	15	20	20	18	24	10	0		34	210	17
Mean	F	36	21	23	20	19	24	27	19	24	15	14	21	30	12	28		27	277	22

TABLE X I Frontal ganglionectomy and faeces production.
Daily weight changes in fourth instars.
Operated Controls.

No.	Sex	Body weight (in mg) on Day: 1	2	3	4	5	6	7	8	9
1	F	235	221	331	379	437	538	637	655	603
2	F	220	204	324	369	445	513	604	610	561
3	F	213	195	322	361	436	523	618	630	584
4	F	226	206	335	391	477	564	600	647	595
5	F	239	217	360	413	485	542	608	609	548
6	F	210	193	341	355	439	491	594	602	556
7	M	219	196	254	345	415	444	506	561	505
8	M	246	225	296	349	428	471	542	521	467
9	M	195	172	247	348	422	459	517	523	468
10	M	196	177	270	310	383	413	476	511	467
11	M	205	171	242	303	358	409	486	461	428
12	M	240	213	277	278	D				

TABLE XI Frontal ganglionectomy and faeces production.

Daily weight changes in fourth instars.

Operated animals: (a) Growth (over 100% increase in weight) and attempted moulting.

No.	Sex	Body weight (in mg) on Day: 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17.
1	F	219	197	263	299	292	273	259	281	340	346	344	420	445	484	436	395	350
2	M	202	182	241	265	292	340	387	438	465	439	394	362					

TABLE XI Frontal ganglionectomy and faeces production.

Daily weight changes in fourth instars.

Operated animals: (b) Reduced growth (50-100% increase in weight) and attempted moulting.

No.	Sex	Body weight (in mg) on Day: 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
3	F	228	204	275	276	265	296	316	296	296	339	306	327	324	340	352	362	348
4	F	272	240	279	280	304	317	361	343	339	380	410	396	407	423	396	370	369
5	M	216	183	249	249	270	339	332	320	294	280	278	258	239				

No.	Sex	Body weight (in mg) on Day: 18	19	20	21	22	23	24
3	F	376	390	407	415	422	379	338
4	F	317						

TABLE XI Frontal ganglionectomy and faeces production.

Daily weight changes in fourth instars.

Operated animals: (c) Reduced growth (50-100% increase in weight) and no attempted moulting.

No.	Sex	Body weight (in mg) on Day: 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
6	F	260	218	295	318	298	342	376	352	398	400	371	321	321	333	336	357	383
7	F	232	209	215	270	286	287	272	288	293	301	313	355	380	344	379	333	322
8	F	210	179	216	277	275	312	324	325	240	268	265	327	328	320	308	273	252
9	M	206	178	263	256	307	311	303	293	274	267	280	268	287	282	248	231	D
10	M	209	188	226	255	295	315	278	302	302	285	260	D					
11	M	200	172	259	271	263	315	330	370	340	319	D						

No.	Sex	Body weight (in mg) on Day: 18	19	20	21	22	23	24	25	26	27
6	F	393	335	364	368	359	331	321	288	256	D
7	F	290	271	D							
8	F	240	D								

TABLE XI Frontal ganglionectomy and faeces production.

Daily weight changes in fourth instars.

Operated animals: (d) Little or no growth (0-50% increase in weight) and no attempted moulting.

No.	Sex	Body weight (in mg) on Day: 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
12	F	253	218	307	299	307	317	339	311	301	299	326	315	310	299	291	D
13	F	247	213	282	306	277	297	317	293	302	275	279	266	269	300	310	D
14	M	198	180	250	261	245	274	247	224	248	258	260	269	236	D		
15	F	237	198	261	306	318	281	256	274	275	288	269	249	D			
16	F	250	214	256	256	264	253	297	320	306	282	269	D				
17	M	197	167	236	213	208	227	226	207	219	205	207	D				

TABLE XI Frontal ganglionectomy and faeces production.

Daily faeces production in fourth instars.

Operated controls.

No.	Sex	Dry wt. of faeces (in mg) on Day 3	4	5	6	7	8	Max. body weight gain (%)	Total weight faeces (mg)	Mean daily faeces production (mg/day)
1	F	19	47	47	48	73	26	179	260	43
2	F	17	47	52	50	72	21	177	260	43
3	F	17	46	65	45	70	30	196	273	46
4	F	20	40	47	36	65	44	186	252	42
5	F	20	50	63	36	53	5	155	227	38
6	F	17	40	46	41	57	24	187	225	38
Mean	F	18	45	53	43	65	25	180	250	42
7	M	8	33	43	37	58	34	156	213	36
8	M	18	40	43	33	45	7	120	186	31
9	M	12	36	52	31	35	4	168	170	28
10	M	12	39	38	37	45	25	161	196	33
11	M	12	35	42	46	35	6	137	176	29
Mean	M	12	37	44	37	44	15	148	188	31

For control and operated groups, operation body weight = 0%.

TABLE XI Frontal ganglionectomy and faeces production

Daily faeces production in fourth instars

Operated animals: (a) Growth (over 100% increase in weight) and attempted moulting

No.	Sex	Dry weight of faeces (in mg) on Day: 3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	Max. body weight gain (%)	Total weight faeces (mg)	Mean daily faeces production (mg/day)
1	F	17	26	18	17	14	22	36	24	18	46	29	35	20	10	-	121	332	24
2	M	9	17	19	23	38	43	28	25	4	-	-					130	206	23

TABLE XI Frontal ganglionectomy and faeces production

Daily faeces production in fourth instars

Operated animals: (b) Reduced growth (50-100% increase in weight) and attempted moulting

No.	Sex	Dry weight of faeces (in mg) on Day: 3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	Max. body weight gain (%)	Total weight faeces (mg)	Mean daily faeces production (mg/day)
3	F	14	13	16	16	14	16	18	23	15	11	15	20	17	17	20	15	15	11	13	14	14	-	85	317	15
4	F	16	13	24	18	26	18	20	30	19	22	16	19	15	9	5	-							56	270	18
5	M	19	25	22	21	15	20	11	9	8	4	-												57	154	15

TABLE XI Frontal ganglionectomy and faeces production

Daily faeces production in fourth instars

Operated animals: (c) Reduced growth (50-100% increase in weight) and no attempted moulting

No.	Sex	Dry weight of faeces (in mg) on Day: 3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	Max. body weight gain (%)	Total weight faeces (mg)	Mean daily faeces production (mg/day)
6	F	17	35	17	20	28	21	25	21	11	10	22	14	13	24	16	24	19	19	20	17	15	10	6	10	D	54	434	18
7	F	13	14	20	17	12	16	15	21	19	29	17	16	22	15	18	10	8	D								64	282	17
8	F	12	30	19	20	26	18	22	28	24	17	19	16	17	12	7	4	D									75	286	18
9	M	9	28	16	24	18	17	15	16	18	14	14	12	10	2	D											51	213	15
10	M	15	13	19	19	18	20	13	6	3	D																51	126	14
11	M	19	23	19	28	19	31	25	10	D																	85	174	22
Mean	M	14	21	18	24	18	23	18	11	11	14	14	12	10	2												62	171	17
Mean	F	14	26	19	19	22	18	21	23	18	19	19	15	17	17	14	13	14	19	20	17	15	10	6	10		64	334	18

TABLE XI Frontal ganglionectomy and faeces production

Daily faeces production in fourth instars

Operated animals: (d) Little or no growth (0-50% increase in weight) and no attempted moulting

No.	Sex	Dry weight of faeces (in mg) on Day: 3	4	5	6	7	8	9	10	11	12	13	14	15	16	Max. body weight gain (%)	Total weight faeces (mg)	Mean daily faeces production (mg/day)
12	F	22	14	21	16	15	17	19	15	18	16	11	11	4	D	34	199	15
13	F	10	23	12	11	26	16	13	9	9	11	20	10	5	D	28	175	13
14	M	19	19	12	12	13	10	8	9	8	9	12	D			38	131	12
15	F	11	24	21	10	8	19	11	10	5	2	D				34	121	12
16	F	11	19	12	7	17	19	8	2	0	D					28	95	11
17	M	14	9	13	11	16	17	13	10	6	D					20	109	12
Mean	M	17	14	13	12	15	14	11	10	7	9	12				29	120	12
Mean	F	14	20	17	11	17	18	13	9	8	10	16	11	5		31	148	13