HISTOLOGICAL STUDIES ON THE BRAIN AND RETROCEREBRAL COMPLEX OF THE COCKROACHES, *PERIPLANETA AMERICANA* L. AND *BLATTA ORIENTALIS* L.

(DICTYOPTERA : BLATTARIA : BLATTIDAE)

T H E S I S

for the

Degree of Doctor of Philosophy

in the

University of Glasgow

by

Tauquirur Rahman Khan, M.Sc.

May, 1962

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

HISTOLOGICAL STUDIES ON THE BRAIN AND RETROCEREBRAL COMPLEX OF THE COCKROACHES, *PERIPLANETA AMERICANA* (L.) AND *BLATTA ORIENTALIS* L.

(DICTYOPTERA : BLATTARIA : BLATTIDAE)

C O N T E N T S

ACKNOWLEDGEMENTS

I am grateful to the University of Glasgow for the financial support afforded for this research. I wish to record my gratitude to Professor C.M. Yonge, C.B.E., F.R.S., for the research facilities put at my disposal.

I am deeply indebted to Dr A. Fraser of the Department of Zoology for suggesting this problem and for the constant encouragement, helpful advice and criticism he has given to me throughout the course of this work. I must also thank Dr A.R. Hill, Dr K.H. Lockey, Dr G. Owen, Mr J.B. Cowey, Mr P.N.R. Usherwood, Mr D.B.C. Scott and Miss J.I. Campbell of the Department of Zoology for their advice and interest in this work.

My thanks are also due to Dr H.F. Steedman for his help in histological technique and to Mr R.A. Crowson for translating Russian, German and Italian papers and to other colleagues who offered advice from time to time.

Finally I am indebted to Mrs P.S. Maitland who made the photographs.

PREFACE

This work is concerned with aspects of the development of the central nervous system and certain endocrine organs, and with the phenomenon of neurosecretion in two species of cockroaches. These insects, Periplaneta americana L., the American cockroach and Blatta orientalis L., the oriental cockroach, are ubiquitous pests which have often been suspected, at least, of being disease carriers (Mallis, 1954). They are quite common in restaurant and hotel kitchens and even in houses in certain parts of the City of Glasgow and in this community alone the cost of control measures runs into several thousands of pounds per year. If any justification is needed for such an apparently academic exercise as this it, lies in the fact that the more that is known about any pest species the easier it may ultimately become to come to terms with it. Furthermore, the lacunae in our knowledge of the anatomy and development of the nervous system of cockroaches which this work attempts to fill should be of value not only to those interested in these particular insects but also to all students of insect anatomy and development since these species are 'used in zoological laboratories as the introductory type exemplifying insect morphology' (Imms, 1951). An additional advantage in using cockroaches as

laboratory material lies, of course, in the fact that both species are relatively easy to rear and maintain in a warm room, the main disadvantage being the relatively long duration of each instar and total life span. In dealing with insects with a short life span histologists have tended to make observations, that is to say kill specimens for sectioning, at relatively short intervals of time but as the duration of instars increases the numbers of observations tend to remain constant and so the interval between observations increases. Thus important changes and cytological phenomena which occur quite rapidly can easily be missed. It is made abundantly clear in the account which follows that a refusal to be daunted by numbers of specimens to be sectioned leads inevitably to rewarding results.

This thesis, which deals with original work undertaken during the years 1959-1962 in the Department of Zoology of the University of Glasgow, has been divided for convenience into four chapters dealing with four aspects of the central nervous system and associated structures as detailed in each chapter heading. The final chapter, which attempts to group and classify the neurosecretory cells of the central nervous system, excluding thoracic and abdominal ganglia, is a response to the refusal of some

neurophysiologists to recognise the fact that an astonishingly high proportion of all neurons may be concerned in endocrine secretion. It also reinforces Fraser's (1957) insistence that all neurosecretory cells of the brain should not be regarded as similar units, functioning synchronously and controlling a single physiological process but rather that the neuroendocrine systems of Insecta are possibly of the same order of complexity as those of Vertebrata.

Finally, the author has naturally been trained to think of cockroaches as belonging to the order Orthoptera but recently authorities (e.g. Richards & Davies, 1957) have agreed to separate cockroaches and mantids from locusts, crickets, etc., and place the former pair in a separate order Dictyoptera, the cockroaches belonging to a suborder Blattaria, family Blattidae, and the mantids belonging to a suborder Mantodea, family Mantidae. This new convention has been adopted in this thesis.

CHAPTER I

THE ANATOMY OF THE BRAIN, SYMPATHETIC NERVOUS SYSTEM AND RETROCEREBRAL COMPLEX IN THE COCKROACH, PERIPLANETA AMERICANA L. AND BLATTA ORIENTALIS L.

INTRODUCTION

The microanatomy of the brain of Periplaneta americana L., has been described by Hanström (1928, 1940), Scharrer (1939), Jawlowski (1948), Drescher (1960), Neder (1960), Willey (1961) and others. That of Blatta orientalis L., has been described by Flögel (1878), Newton (1879), Haller (1905), Brètschneider (1914), Sanchez (1933), Neder (1960) and others. The earliest descriptions of what is now known as the stomatogastric nervous system in a cockroach (B. orientalis) are attributable to Newton (1879) and Hoffer (1887) but these are now of little more than historical value. The anatomy of the stomatogastric nervous system (stomodeal nervous system) of cockroaches, and of other insects, was outlined by Snodgrass (1935, 1943) and has been treated in some detail recently by Drescher (1960) and Willey (1961). The retrocerebral complex of insects, which is intimately associated with the brain and stomatogastric nervous system, has been described by Hanström (1940), Cazal (1948), Enbohm (1948), Drescher (1960), Harker (1960), Willey (1961) and others.

The observations of Nesbitt (1941) on Blaberus, of Füller (1960) on P. americana and of Highnam (1961) on Schistocerca, are useful in enabling one to resolve some problems in the interpretation of the anatomy of the retrocerebral complex.

Despite the large volume of previous publications on this subject the present work is justified on the grounds that such studies are essential preliminaries to the study of the embryonic development of the brain in cockroaches and to studies of neurosecretion and related phenomena. These investigations were completed before the most recent workers, i.e. Drescher (1960), Füller (1960), Harker (1960), Neder (1960) and Willey (1961), noted above, had published their results so that the author had arrived at his conclusions independently and, as will be seen later, this account now provides an opportunity to correct certain errors and resolve disagreements in previously published works on this subject.

MATERIAL AND METHODS

Heads of twenty specimens of P. americana, last instar nymphs and adults of both sexes, were dissected in insect Ringer's solution under a binocular microscope. When the tissues overlying the brain and frontal ganglion had been removed the dissection was flooded with either methylene blue or Delafield's haematoxylin or 0.5 per cent iron

haematoxylin. The stain was applied by means of a fine glass pipette in the vicinity of the frontal ganglion, brain, suboesophageal ganglion and retrocerebral complex. In addition, serial sections of the heads of nymphs and adults of P. americana and B. orientalis prepared by the methods described in Chapter 3, were also studied to substantiate the results obtained by dissection.

With Delafield's haematoxylin the outer surface of the ganglia and the nerves stain in various shades of purple, the connective tissue and muscle fibres being purple to black and the tracheal epithelium purple. The presence of the shining linings of trachea helps one to distinguish these from other structures. With methylene blue the ganglia and the nerves stain blue and with iron haematoxylin blue-black.

OBSERVATIONS

Section I: The Brain

I(a) Structure of the brain of P. americana and B. orientalis

The brain of a first instar nymph is more or less oval in shape and fills almost the entire head capsule. Initially there is very little space between the neural lamella which covers the brain and the head capsule but in later instars the space increases to accommodate the

developing head muscles and connective tissues. The external form of the brain approximates more closely to that of the adult in successive instars.

The brain can be divided into three regions named the protocerebrum, deutocerebrum and tritocerebrum. In a frontal section, the brain is seen to be covered with an outer neural lamella within which lies the layer formed by perineurium cells. The brain contains cortical layers of nerve cells of different sizes most of which are thought to be association neurons (Snodgrass, 1935). The medulla of the brain consists of a neuropile with nerve tracts and denser association centres. Glia cells, currently supposed to be concerned with the transfer of nutritive material to neurons (Wigglesworth, 1959, 1960), fill the spaces between neurons in the cortex and are scattered in the neuropile.

I(b) The Protocerebrum (Figure 1)

The protocerebrum is antero-dorsal and forms the largest part of the brain. The two rounded masses of the protocerebral lobes are partially separated by the median fissure. The two occellar nerves arise from the front of the mid-dorsum of the protocerebrum and go to the so-called femestrae at the bases of the antennae. The optic lobes project from either side of the protocerebrum. The

Figure 1. Diagram of the protocerebrum of P. americana protocerebral lobes (PL), median fissure (MF), ocellar nerves (ON), optic lobes (OPT), pons cerebralis (PC), central body (CB), ventral mass (VM), calcices (CX), peduncles (Ped), trabeculae (Tr), cauliculi (Cau), medulla externa (ME), medulla interna (MI), external chiasma (ECH), internal chiasma (ICh), optic nerve (OptN), post-retinal fibres (PRF).

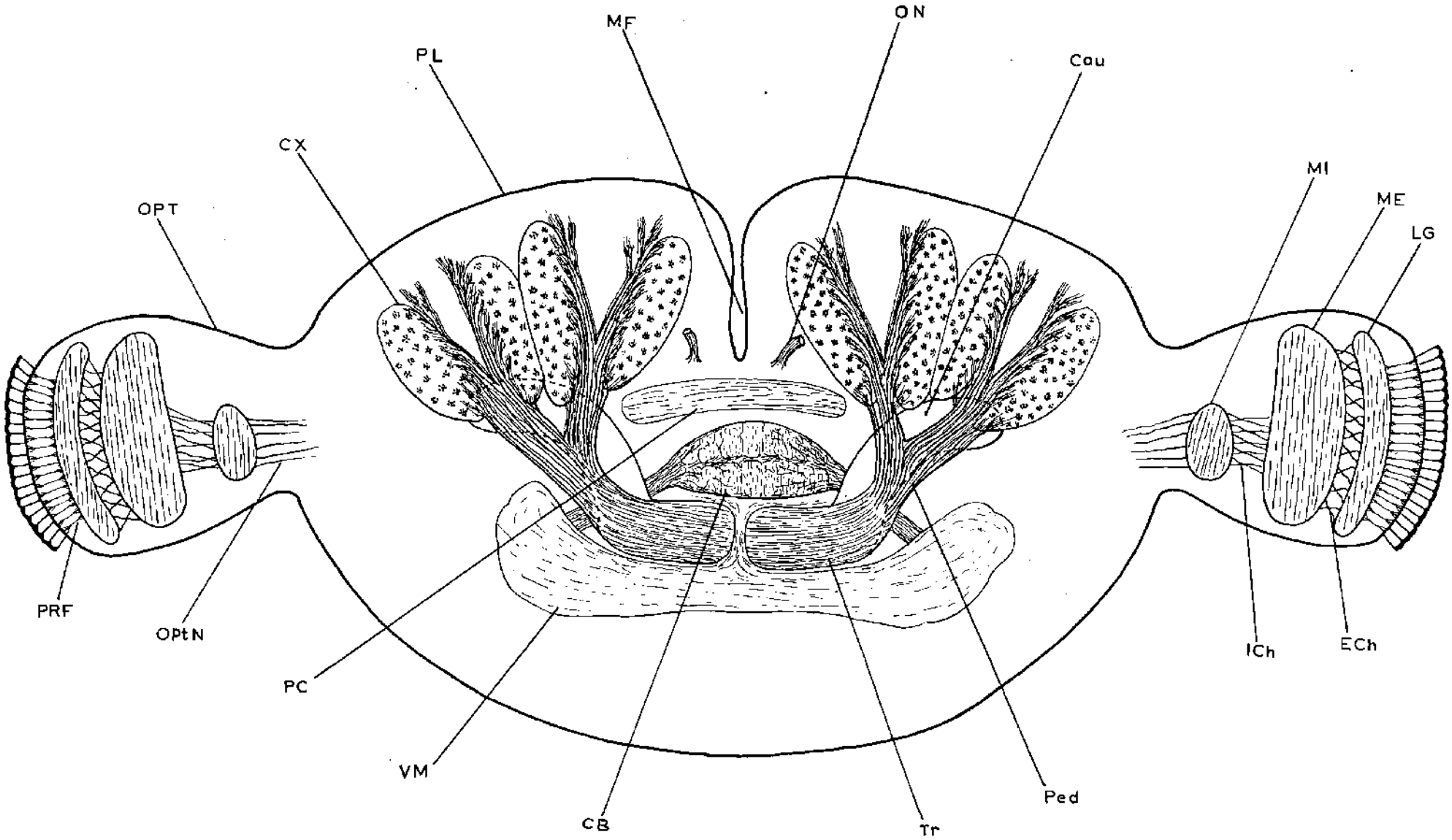


Figure 1.

protocerebrum contains a number of important association centres, namely the corpora pedunculata (mushroom bodies), pons cerebralis, central body, ventral mass (corpus ventrale) and its ventral protocerebral commissural tract. The corpora pedunculata (CP) lie on the antero-dorsal side of the protocerebrum and are most conspicuous structures. Each CP consists of an outer and an inner cup or calyx filled with numerous association neurons known as globuli cells, a peduncle or stalk connected with each calyx and inserted on the inner side of the trabecula, a cauliculus inserted on the outer side of the trabecula and the trabecula itself. The trabecula curves inwards and in some specimens the free end of the trabecula of one half of brain is in direct contact with the free end of the trabecula of the other half but in most cases they are separated and the only connecting link is the subtrabecular-commissure (Drescher, 1960). The cauliculus is curved. Its free end may sometimes almost reach the neural lamella of the lateral part of the protocerebrum. The two calices of the mushroom body lie at the outer convex anterior part of the cauliculus and are connected with the cauliculus by nerve fibres. The globuli cells of the CP are very poor in cytoplasm, almost the entire volume of the cell being occupied by the nucleus

which is rich in chromatin material. In anterior sections the calices of the CP appear as half moon shaped structures with small glomeruli, i.e. synaptic junctions, in their main body and are surrounded by nerve fibres. The glomeruli are clearly visible after both silver and haematoxylin stains. The dendrites of the globuli cells lie in the calcies and synapse with nerve fibres coming from different parts of the brain and from the ventral nerve cord via the suboeso-:phageal ganglion to form these small glomeruli (Du Porte, 1959). In each calyx of the CP two groups of globuli cells can be seen and, in consequence, in the posterior sections when each calyx seems to have split up into two, one can see two groups of axons of globuli cells running along the inner lateral part of each split part of a calyx. In each half of the protocere-:brum the two large axon groups unite to form the common stalk of the mushroom body. The stalk, the cauliculus, and the trabecula are formed by the axons of the globuli cells lying in the calices. Individual axons within the stalk can be seen but it is difficult to trace single nerve fibres within the fine dense neuropile of the cauliculus and trabecula.

The pons cerebralis (or protocerebral bridge) is

a transversely elongated structure lying antero-dorsal to the central body and posterior to the pars intercere-:bralis. The pons cerebralis appears to be an association centre since fibres enter it from various regions of the brain (Snodgrass, 1935). The pars intercerebralis is that part of the brain, bounded by the corpora pedunculata, which lies between the two protocerebral lobes (Haller, 1905). The outer part of a protocerebral lobe lateral to the corpus pedunculatum is called the pars lateralis. Most of the neurosecretory cells of the brain are found in the pars intercerebralis though the pars lateralis also contains some neurosecretory cells (see Chapters 3 and 4). The central body lies just above the inner end of the trabecula and ventral to the pons cerebralis. Fibres from all regions of the brain converge in the central body. The two protocerebral lobes are interconnected by the central body.

The two ventral masses (or Neben lappen of German authors) lie in ventro-lateral position in the proto-:cerebral lobes and each is connected to that of the other side by a transverse band of nerve fibres lying immediately beneath the trabeculae. These association centres are connected by nerve fibres with the other association centres as well as with the other parts of the brain.

The optic lobe (Figs. 1, 2) lies on the lateral side

Figure 2(a). Photomicrograph of a frontal section of the optic lobe of an adult P. americana, indicating lamina ganglionaris (LG), medulla externa (ME), medulla interna (MI), globuli cells (GC), position of external chiasma (Ech) and internal chiasma (Ich).

Figure 2(b). Photomicrograph of a following frontal section of the optic lobe of the same specimen. Note the optic nerve (OPtN) and the large neurons (N).

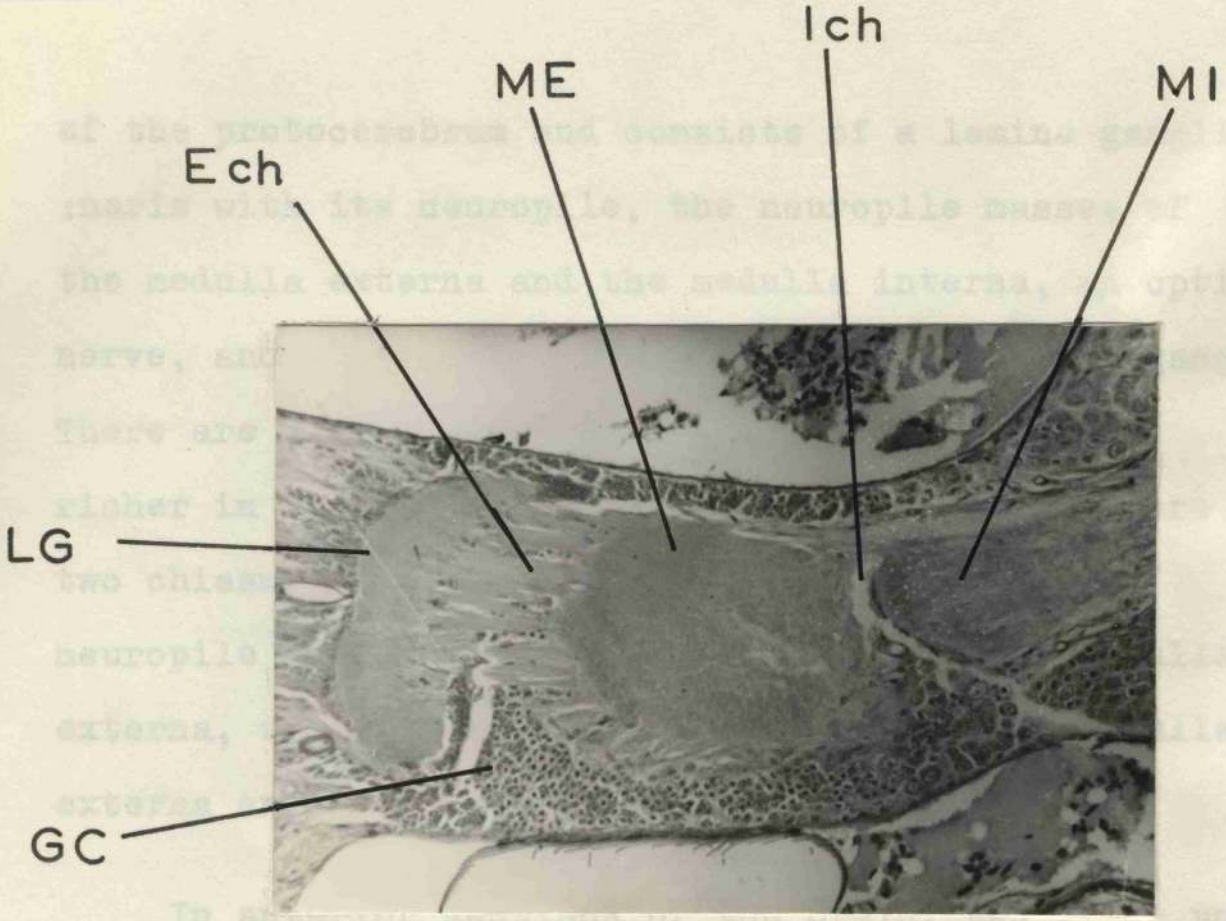


Figure 2(a).

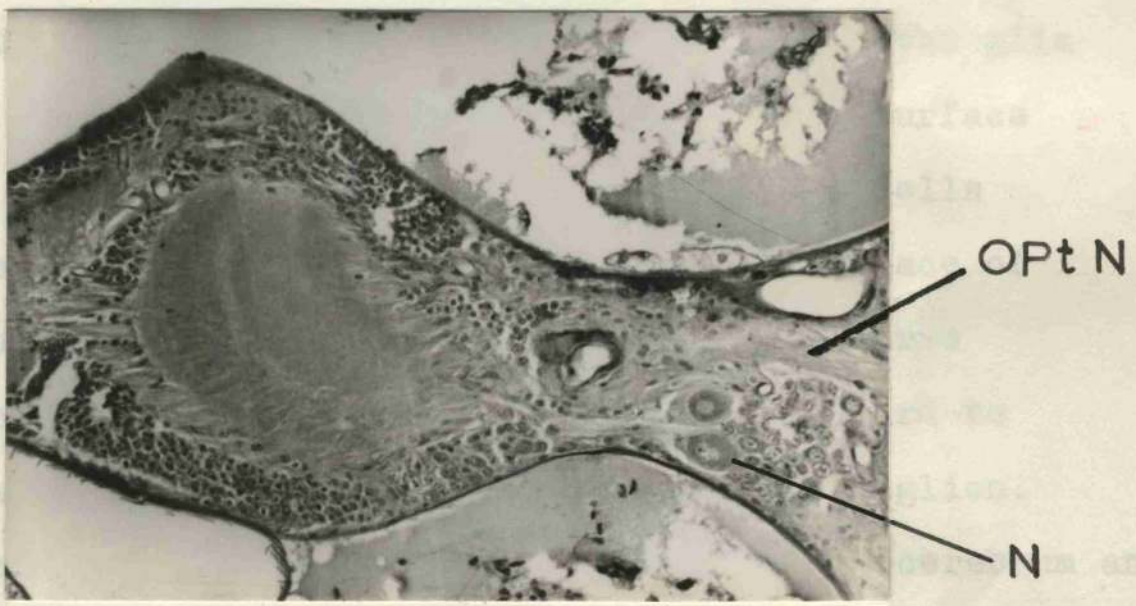


Figure 2(b).

of the protocerebrum and consists of a lamina ganglio-:naris with its neuropile, the neuropile masses of the medulla externa and the medulla interna, an optic nerve, and the small globuli cells of the optic ganglion. There are other neurons also which are bigger and richer in cytoplasm than the globuli cells. There are two chiasmata, an external chiasma between the neuropile of the lamina ganglionaris and the medulla externa, and an internal chiasma between the medulla externa and the medulla interna.

In anterior sections of the brain, two large unipolar neurons (Fig. 3a and b) can be found on the ventral side of the protocerebrum close to the perilemma and there is a group of nerve cells lying in a half circle and more or less encircling them but separated from them by the glia cells. Glia cells are closely applied to the surface of these cells. The thick axons of these two cells penetrate the neural lamella on the ventral surface of the protocerebrum and emerge to form a median nerve called the nervus connectivus which passes forward to enter the postero-dorsal part of the frontal ganglion. This, the only connecting link between the protocerebrum and frontal ganglion, was first named by Baldus (1924) who found it in a dragon fly, Aeshna. Bretschneider (1914) saw this structure in B. orientalis but regarded it as

Figure 3a. Photomicrograph of a frontal section of anterior portion of the protocerebrum, showing two large nervus connectivus neurons (N) and their tubular axons which form the nerve called the nervus connecti-:vus (NC). The nervus connectivus has penetrated the neural lamella of the protocerebrum to go to the frontal ganglion. Note also the glia cells (G) which separate the nervus connectivus cells from the para connectivus neurons (see Figures 81 and 82). Adult ♂ P. americana killed less than 1 hour after moulting.

Figure 3b. Photomicrograph of a frontal section of the frontal ganglion, showing the nervus connectivus lying in the tube formed by an extension of the posterior dorsal part of the ganglion. Note also the nervus connectivus entering the neuropile of the frontal ganglion (FG). Same specimen as above. Lettering as in Figure 3a.

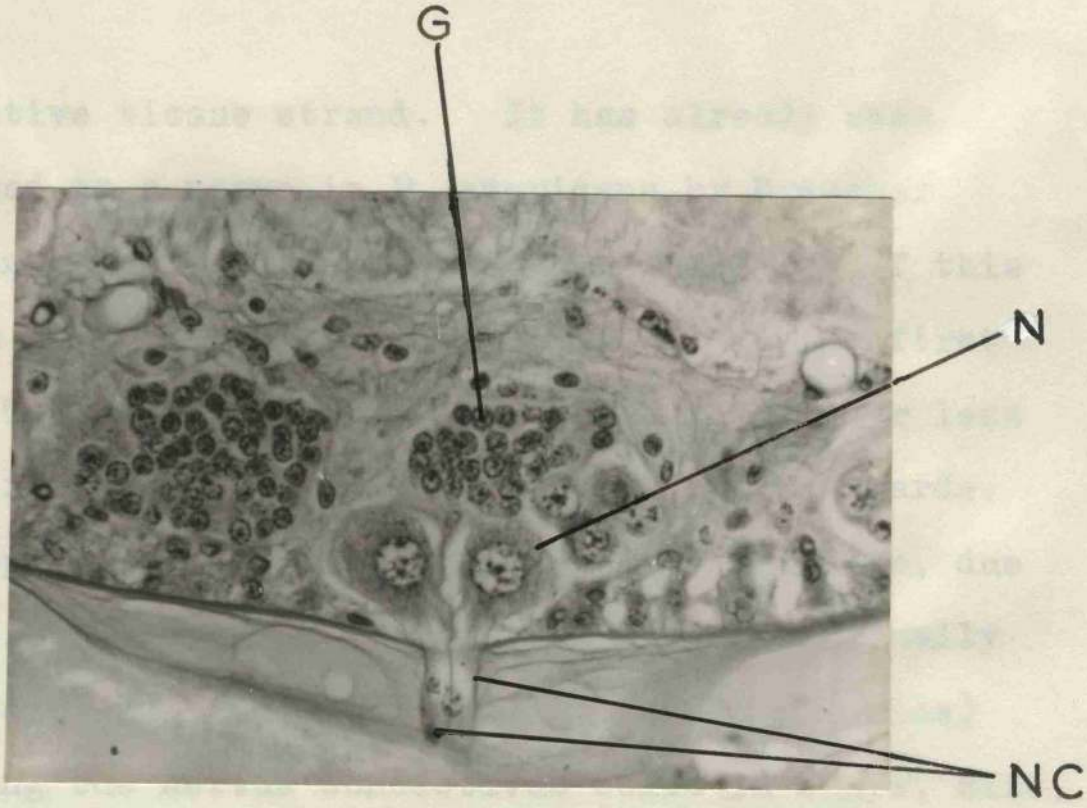


Figure 3(a).

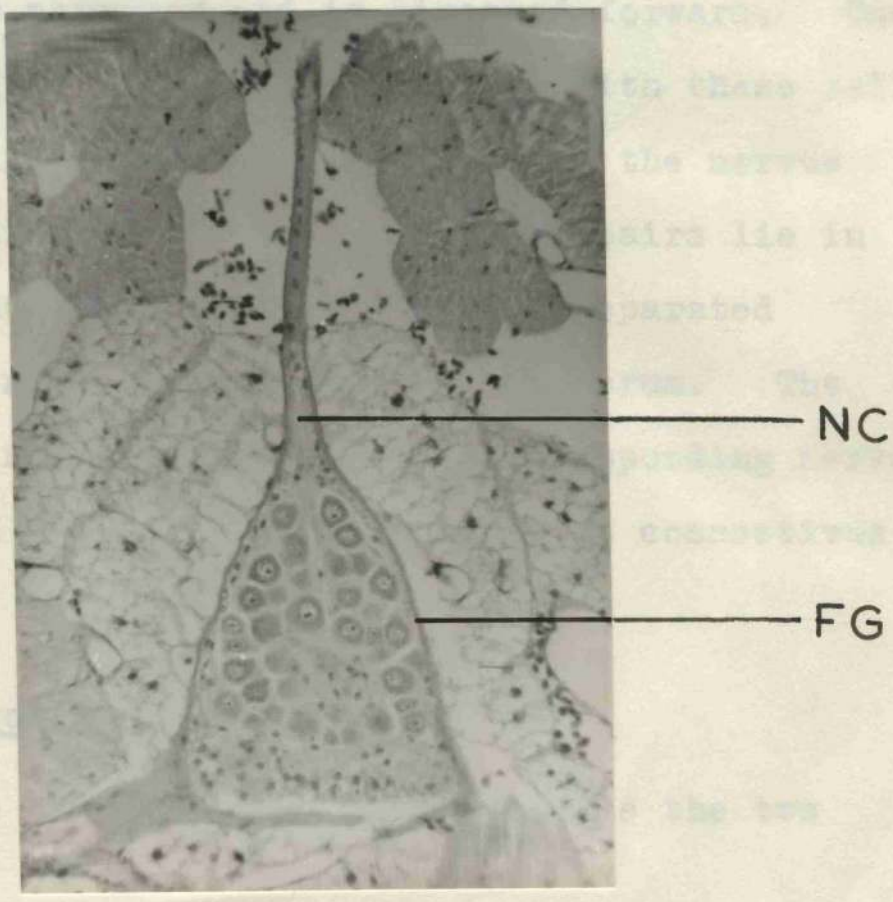


Figure 3(b).

a connective tissue strand. It has already been recognised as a nerve in P. americana by Drescher (1960) and by Willey (1961) and the existence of this nerve in B. orientalis is now recorded for the first time. The nervus connectivus cells are more or less conical in shape, the narrow end pointing downwards. The cytoplasm may appear 'spongy' after fixation, due to the presence of ill-defined vacuoles, and usually stains poorly. Two other cells (one on each side) resembling the nervus connectivus cells in shape, size, and staining properties, can be seen in the median pars intercerebralis, just below the dorsal perilemma of the protocerebrum and near the median fissure of the brain. Their narrower end is directed forward. One or two glia cells are in close contact with these cells also. The similarity between these and the nervus connectivus cells is remarkable. Both pairs lie in the same horizontal plane but they are separated widely by the neuropile of the protocerebrum. The second two do not appear to form a corresponding nerve. The functional significance of the nervus connectivus is unknown.

I(c) The Deutocerebrum (Figure 4)

Below the two protocerebral lobes lie the two

Figure 4. Photomicrograph of a frontal section of deutocerebrum of P. americana, indicating antennal lobes (AL), antennary nerves (AN), transverse commissure (TC), neurons (N), neuropile (NP), glomeruli (GL).

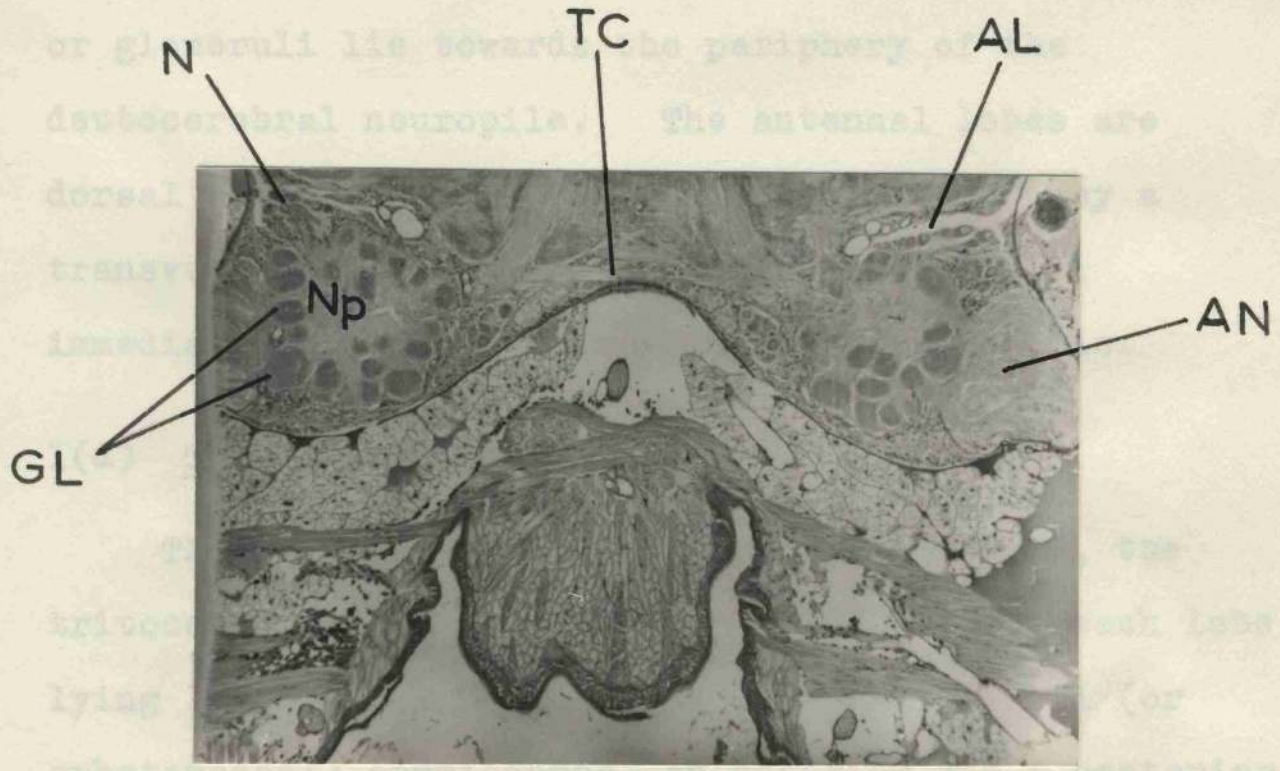


Figure 4.

smaller antennal lobes which form the deutocerebrum. Arising from the antennal lobes are the antennary nerves. The neurons of the deutocerebrum lie towards the periphery, the central part consisting of the neuropile of the antennal lobe. The synaptic areas or glomeruli lie towards the periphery of the deutocerebral neuropile. The antennal lobes are dorsal to the gut and are connected together by a transverse commissure lying above the gut and immediately beneath the protocerebral neuropile.

I(d) The Tritocerebrum (Figure 5)

The third and smallest part of the brain, the tritocerebrum, lies below the deutocerebrum, each lobe lying lateral to the gut. Two suboesophageal (or substomodeal) commissures, an anterior and a posterior one transversely connect the tritocerebral lobes below the stomodaeum. Such a connection has also been noted by Willey (1961). The anterior suboesophageal commissure is thin, slender and longer than the posterior one and can best be observed in frontal sections. The posterior one is thick, broad and short and lies just above but separated from the anterior part of the suboesophageal ganglion. The circum-oesophageal connectives arise from the posterior end of the tritocerebrum and run posteriorly and ventrally to the

Figure 5. Diagram of tritocerebrum of P. americana indicating tritocerebral lobes (TC), anterior suboesophageal commissure (ASC), posterior suboesophageal commissure (PSC), circum-oesophageal connective (COC), NCCIII, tegumentary nerves (TN), labro-frontal nerve (LFN), suboesophageal ganglion (SOG), labral nerve (LbN), frontal connective (FC), neurons (N), neuropile (Np), a portion of the deutocerebrum (DC).

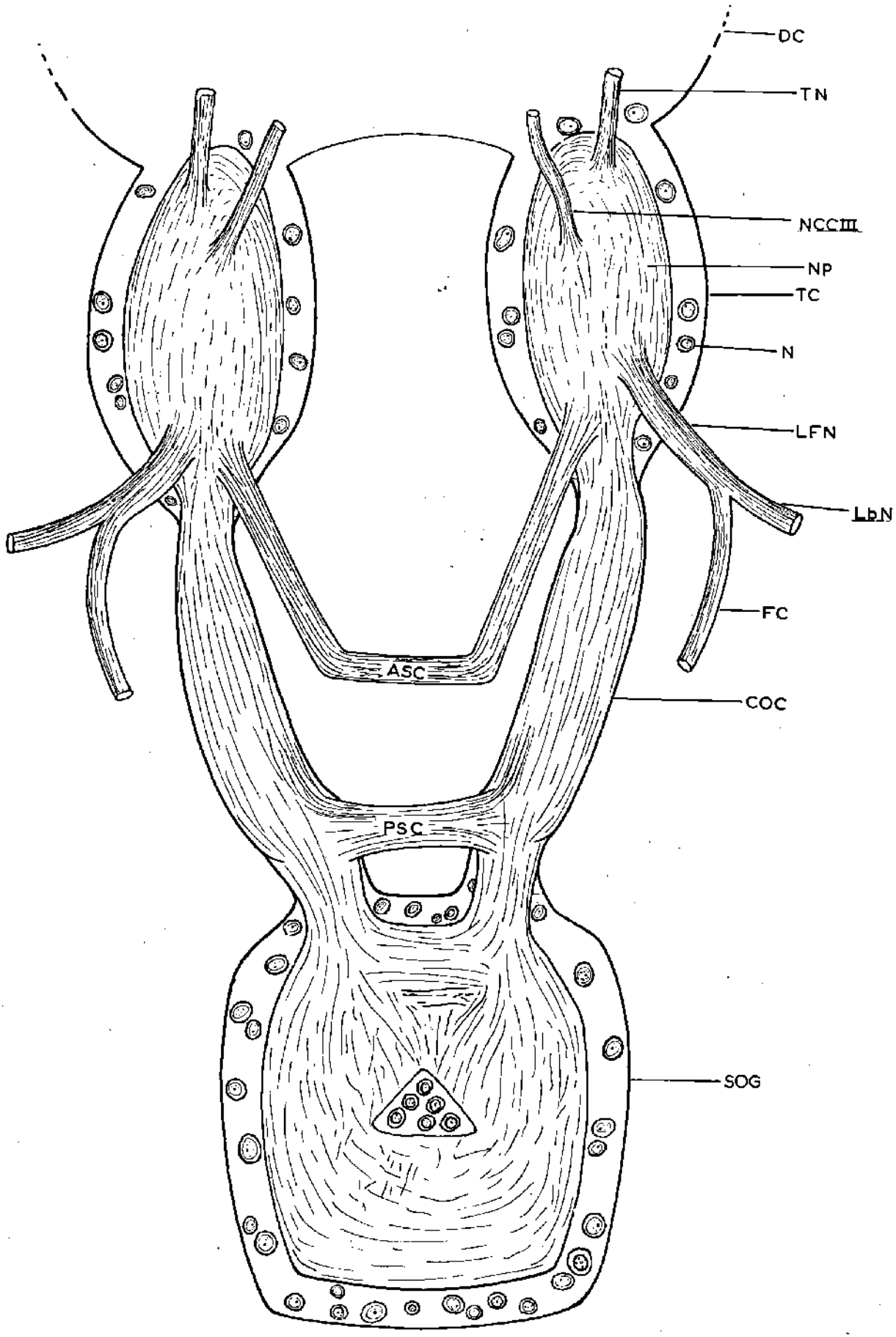


Figure 5.

suboesophageal ganglion which they join at its anterior end. From the inner antero-lateral faces of each tritocerebral lobe there emerges a nerve which passes upwards to unite with the corpus cardiacum. These two nerves are referred to as the nervi corporis cardiaci III (NCCIII), (Dupont-Raabe, 1956). Another pair of nerves, the tegumentary nerves, arise from the antero-dorsal faces of the tritocerebral lobes, these going to the dorsal part of the head. The common root of the labral nerve and the frontal connective emerge from the anterior face of the tritocerebral lobe, the labial nerve going to the labrum and the frontal connective to the frontal ganglion. The neuropile of the tritocerebrum is less glomerular than that of the deutocerebrum.

Section II: The Suboesophageal Ganglion of P. americana and B. orientalis (Figure 6)

Like the brain this is also a composite ganglion. It lies below the brain and ventral to the gut. The mandibles, maxillae, labium, hypopharynx and some of the neck muscles are innervated by the nerves arising from the suboesophageal ganglion. In addition to these it also sends a paired nerve, to be described later in greater detail, from its anterior end to the corpora

Figure 6. Diagram. Lateral view of the suboesophageal ganglion (SOG) of P. americana indicating mandibular nerve (MN), hypopharyngeal nerve (HN), maxillary nerve (MaxN), labial nerve (LN), NCAII, nerves to prothoracic glands (NPG), circum-oesophageal connective (COC), longitudinal commissure (LC).

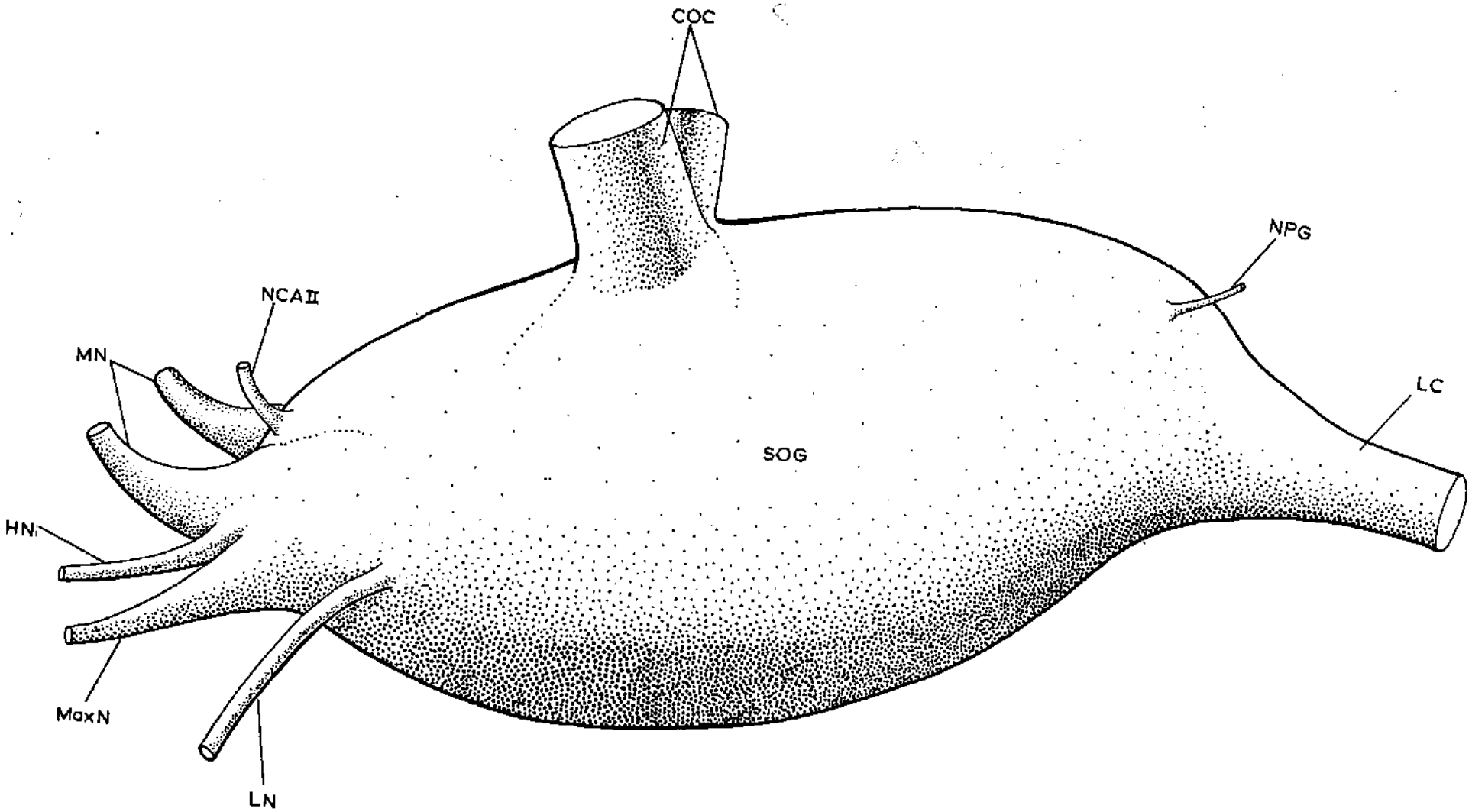


Figure 6.

allata. The presence of this pair was noted by Engelmann (1957) in Leucophaea and confirmed in P. americana by Willey (1961) who has termed them the nervi corporis allati II (NCAII). Their occurrence in B. orientalis is now recorded here for the first time. Another pair of slender nerves arising from the posterior dorsal surface of the suboesophageal ganglion seems to innervate the prothoracic glands. Such a connection has also been reported by Willey (1961) in P. americana. The majority of the neurons lie in the ventral part of the ganglion but those whose axons form the labral tract lie in the central part (Willey, 1961). There are few neurons on the anterior and lateral sides of the ganglion. Most of the central space within the ganglion is occupied by the neuropile. Glia cells can also be observed between the neurons and in the neuropile.

Section III: Stomatogastric (or Stomodaeal) Nervous System of P. americana (Figure 7)

III(a) Frontal ganglion and its nerves

The frontal ganglion, in both species, is more or less triangular in shape. It is situated towards the anterior end of the head, in front of the brain, and

Figure 7. Diagram of the stomatogastric nervous system and retrocerebral complex of P. americana. For explanation of symbols see text.

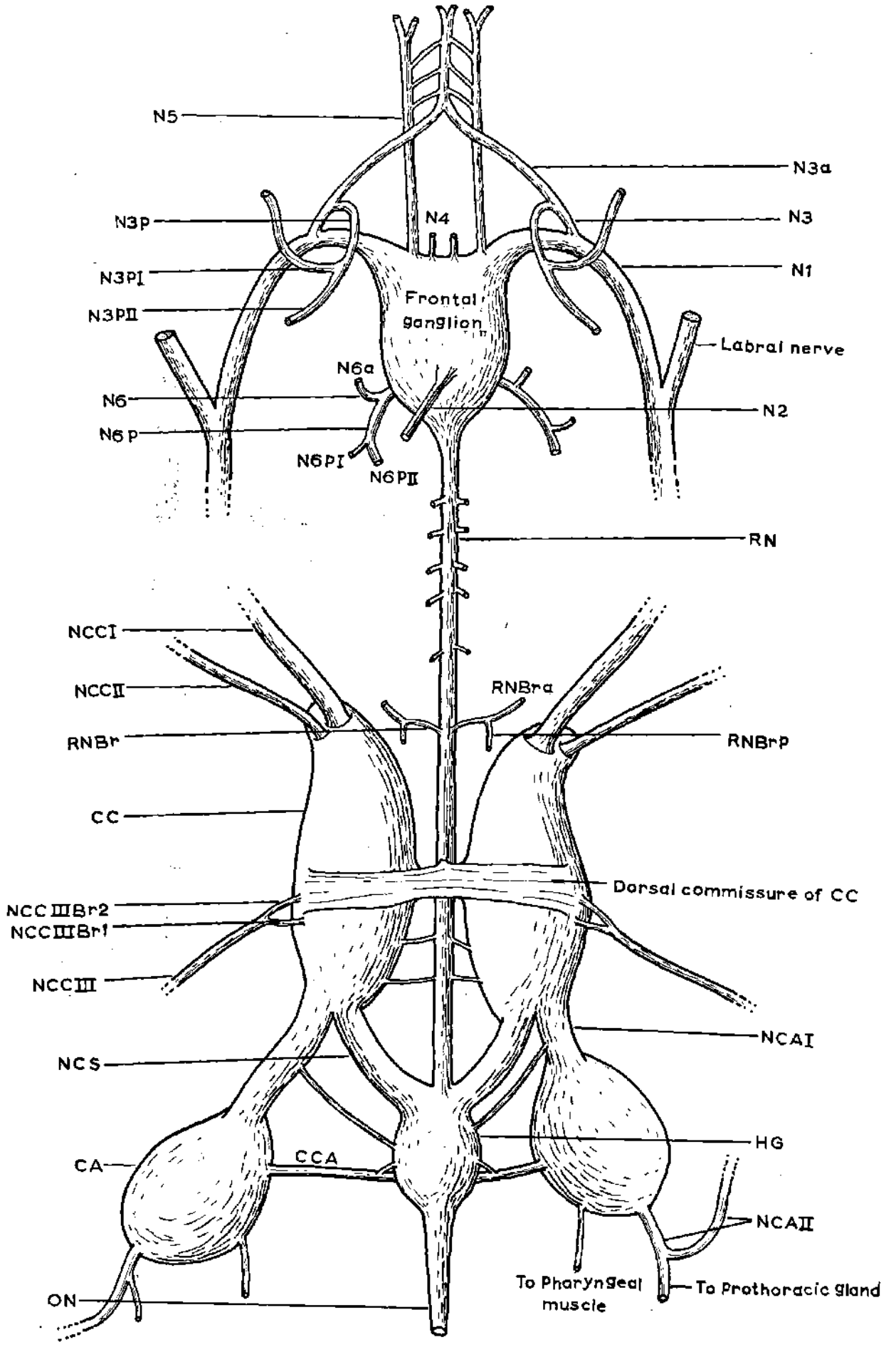

N5
N3a
N3p
N4
N3
N3PI
N1
N3PII
Frontal ganglion
Labral nerve
N6a
N6
N2
N6p
N6PI
N6PII
RN
NCCI
NCCII
RNBra
RNBr
RNBrp
CC
Dorsal commissure of CC
NCCIIIBr2
NCCIIIBr1
NCCIII
NCAI
NCS
HG
CA
CCA
NCAII
To Pharyngeal muscle
To Prothoracic gland
ON

Figure 7.

lies on the dorsal side of the pharynx. Two thick nerves (N_1), looking like horns, project from its antero-lateral margins and curve downwards. These are the frontal connectives which unite the frontal ganglion with the tritocerebral lobes of the brain. As noted above they emerge from the tritocerebrum although according to Willey (1961) they do not originate in that part of the brain but only form synapses therein.

A connection between the hind part of the protocerebrum and the postero-dorsal part of the frontal ganglion is established by a delicate nerve of only two axons known as the nervus connectivus (N_2). The nervus connectivus lies in the tube formed by an extension of the posterior dorsal part of the frontal ganglion running towards the protocerebrum. The two large neurons of the nervus connectivus have already been described above. From the dorsal side of the bend of each of the thick frontal connectives a nerve (N_3) originates and immediately divides into two, forming an anterior branch (N_3a) and a posterior branch (N_3p). The anterior branch forms an arch in front of the frontal ganglion and joins with its fellow of the opposite side in front of the ganglion and this united nerve goes forward and branches to innervate the lateral retractor muscles of the labrum. It also sends fine

branches to the clypeus. The posterior branch (N_3p) which lies above the bend of N_1, again divides into two, an anterior branch (N_3pI) innervating the lateral retractor muscles of the labrum and a posterior branch (N_3pII) innervating the dilator muscles of the pharynx. These branches are fine and great care must be taken in disclosing them by dissection. The presence of connective tissue, muscles and tracheae in close association with the frontal ganglion and its nerves makes the task of tracing these nerves most difficult. Two pairs of nerves arise from the anterior margin of the frontal ganglion, a small anterior median pair (N_4) and two larger nerves (N_5) each of which lies between the frontal connective and the adjacent member of the median pair of its own side. The small N_4 nerves innervate the pharyngeal muscles which lie just below the frontal ganglion. The larger nerves run under the two arches of the labroclypeal nerve and innervate the buccal lining and, on their way, each gives off lateral branches which go off to unite at intervals with the single anterior median labroclypeal nerve, producing a ladder-like appearance. A posterior pair of nerves (N_6) arises from the ventro-lateral side of the frontal ganglion. When viewed from the dorsal side they look small at their point of origin. However, each of these

divides into two nerves, a short thin anterior nerve (N_6a) which meets a trachea and bends downwards and disappears in the precerebral dilator muscles (No. 6 of Snodgrass, 1943) and a larger posterior nerve N_6p (= N_5 of Willey, 1961). The larger branch (N_6p) then divides into a thinner (N_6pI) and a thicker nerve (N_6pII), the former certainly innervating the precerebral dilator muscles (No. 7 of Snodgrass, 1943) of the pharynx but the latter's destination is still uncertain.

Histological examination of the frontal ganglion shows that there are two or three layers of cortical nerve cells and a medullary neuropile made up of interwoven nerve fibres. Trachea and tracholes penetrate the ganglion. There is a distinct perilemma around the ganglion and glia cells can be recognised in the neuropile.

III(b) The recurrent nerve

The recurrent nerve (RN) arises from the posterior limit of the frontal ganglion and passes beneath the brain and above the gut giving off small branches from its ventro-lateral and ventral surfaces to the tunica muscularis of the pharynx and the dilator muscles of the pharynx. It is considered by the author to extend as far as the posterior limit of the corpora cardiaca.

Of the several nerves originating from the recurrent nerve, one pair is worth mentioning. This consists of two slender nerves (RNBr) arising from the ventro-lateral sides of the recurrent nerve. Each runs horizontally for some distance and then divides into two, an anterior branch (RNBra) which curves upwards towards the nervus corporis cardiacum interni (NCCI) which is just entering the corpus cardiacum, and a posterior branch (RNBrp) which bends downwards and appears to innervate the tunica muscularis of the pharynx. The anterior branch, RNBra, and the branch of the NCCI, (NCCI, Br2) to be described later, run side by side anteriorly towards the brain (the last portion of the deutocerebrum or the anterior portion of the tritocerebrum) but their further course is obscured by the dilator muscles of the pharynx. At least two other pairs of fine nerves arise from the ventro-lateral sides of the recurrent nerve and innervate the posterior part of the corpora cardiaca ventrally.

In frontal sections the anterior portion of the recurrent nerve, which is narrow and oval, lies dorsal to the gut and between the widely separate NCCI but subsequently it becomes slightly broader and lies below the corpora cardiaca. The recurrent nerve has

a thin neural lamella overlying the perineurium which contains perineurium cell nuclei. These closely resemble and could be confused with the glia cell nuclei which occur close to the perineurium and dispersed among the nerve fibres. Neurons occur intermittently throughout the entire length of the recurrent nerve. The axons in the recurrent nerve appear to come from the frontal ganglion.

III(c) The hypocerebral ganglion

Towards its posterior end the recurrent nerve widens to become the hypocerebral ganglion (HG). The anterior limit of this ganglion lies at the same level as the anterior limit of the corpora allata (CA). It lies above the gut between the two CA and extends posteriorly, but rarely beyond the CA. Finally it becomes narrow and thereafter continues as the oesophageal nerve (ON) which passes back along the dorsal surface of the oesophagus. The hypocerebral ganglion is considered later as a member of the retrocerebral complex.

Section IV: The Retrocerebral Complex in Cockroaches

(Figure 7)

IV(a) General

Dissection shows the presence of a pair of spindle shaped corpora cardiaca (CC) behind the brain and in close

association with the aorta (Ao). In freshly dissected specimens the CC are bluish-white in colour. Medially the corpora cardiaca are united. Two pairs of nerves, nervi corporis cardiaci interni (NCCI) and nervi corporis cardiaci externi (NCCII) which will be described in detail later, coming from the median and the lateral parts of the protocerebrum respectively, unite the brain and the CC. Each CC receives at its anterior end the NCCI and NCCII of its own side. Each CC receives another nerve, (NCCIII) noted earlier, arising from the tritocerebrum. Behind each CC and connected with its hind end lies a corpus allatum (CA). Between the two CA lies the hypocerebral ganglion as noted above. The two CA are themselves connected together by the commissure of the CA, and the CA are also linked by nerves to the suboesophageal ganglion, the NCAII (Willey, 1961).

IV(b) Nervous connections and structure of the corpora cardiaca

IV(b)1. NCCI. The NCCI are formed by the axons of neurosecretory cells (NSC) of the pars intercerebralis. The pre-chiasmatic NCCI has two distinct roots, an inner coming from the NSC closest to the median groove and an outer which travels for a short distance over the antero-dorsal surface of the protocerebral neuropile (Figs 58,59)

Axons in each of these roots contain neurosecretory granules (see Chapter 3). There is no evidence of a contribution of nerve fibres to the pre-chiasmatic NCCI from the neuropile of the protocerebrum as claimed by Willey (1961). The two NCCI cross in the median dorsal region of the protocerebrum (see Chapter 3) and each continues its course in the opposite half of the brain to that of its origin. They then become straight and run posteriorly, parallel to one another, passing between the trabeculae. Beyond the trabeculae they diverge and turn downwards to emerge from the postero-ventral faces of the protocerebrum after piercing the neural lamella of this region. Willey (1961) has suggested that only half of the fibres of the NCCI cross to the other side of the brain but the author agrees with the observation of Highnam (1961), on Schistocerca, that most of the fibres do cross over. It should be noted that all the axons of the pre-chiasmatic or post-chiasmatic NCCI do not possess neurosecretory material. For this there are two possible explanations, either that NS cells do not all discharge NS material at the same time or that some of the axons in the NCCI belong to neurons of the pars intercerebralis, which are not neurosecretory. Only the latter explanation is

Figure 8. Photomicrograph showing the common origin of three branches (Br1,2,3) of NCCI. The NCCII can also be seen.

Figure 9. Photomicrograph of the section following that in Figure 8. In this Br1 has disappeared but Br2 and Br3 can still be seen. Br3 is clearer in this section than in the preceding one. Note also the NCCII.

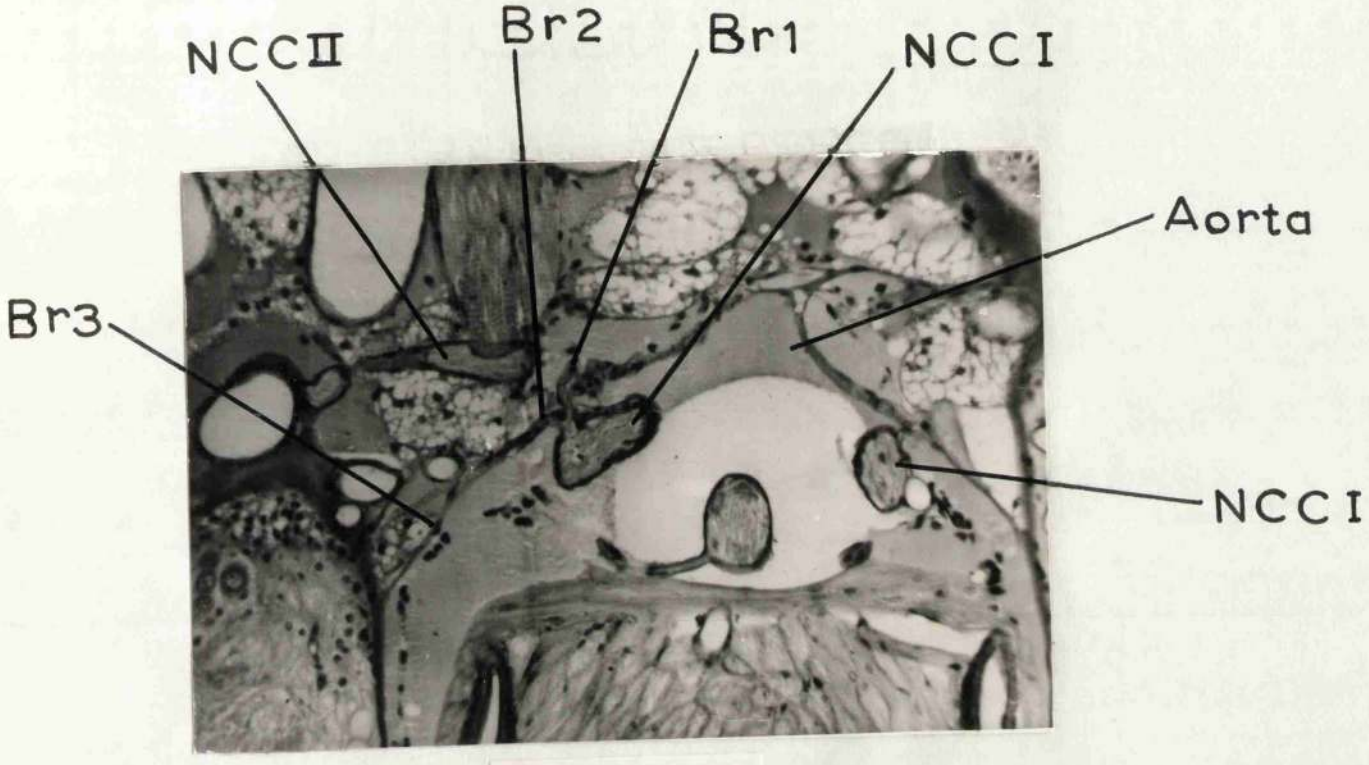


Figure 8.

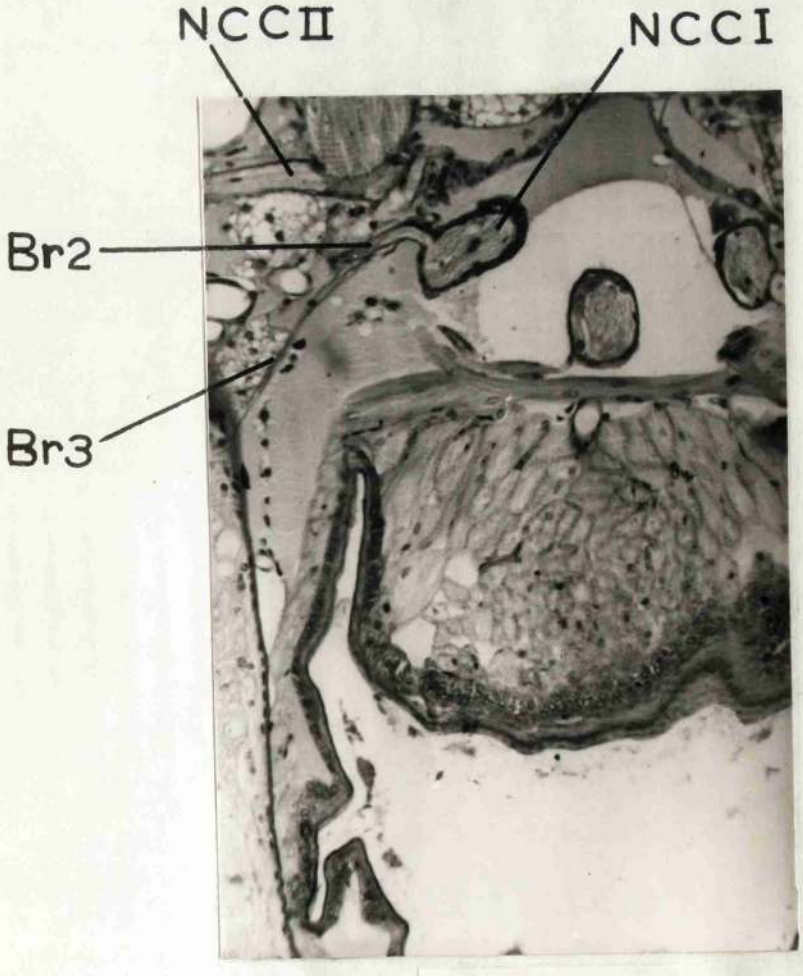


Figure 9.

supported by Stumzollinger (1957), Johansson (1958) and Highnam (1961) although it is quite reasonable to suppose that both are true.

Just prior to its point of junction with the CC each NCCI gives off three nerves which have not previously been described (Figs 8 and 9). The first, a thick nerve (NCCI:Br1), appears to innervate the wall of the aorta. The second, also a thick nerve (NCCI:Br2), goes towards the anterior end of the last portion of the deutocerebrum (or the anterior postion of the tritocerebrum) but its further course is obscured by the sudden appearance of dilator muscles of the pharynx lying above it and by a trachea lying anterior to it. These two nerves arise from the same point on the NCCI, by piercing the perilemma of the NCCI and present a bifurcated appearance at the point of their origin. Their nerve fibres are continuous with those of the NCCI. A third nerve (NCCI:Br3) which is very thin also arises from the NCCI. It lies ventral to the Br2 nerve and its thin finger-like branches penetrate the anterior inner perilemma of that part of the brain which lies antero-lateral to the gut.

IV(b)2. NCCII. The NCCII are formed by the axons of

Figure 10. Photomicrograph of a section through the anterior part of the corpus cardium (CC) of a freshly moulted nymph of P. americana, showing the crossing of nerve fibres of the NCCI and NCCII at the point of their entry into the corpus cardiacum. RN, recurrent nerve.

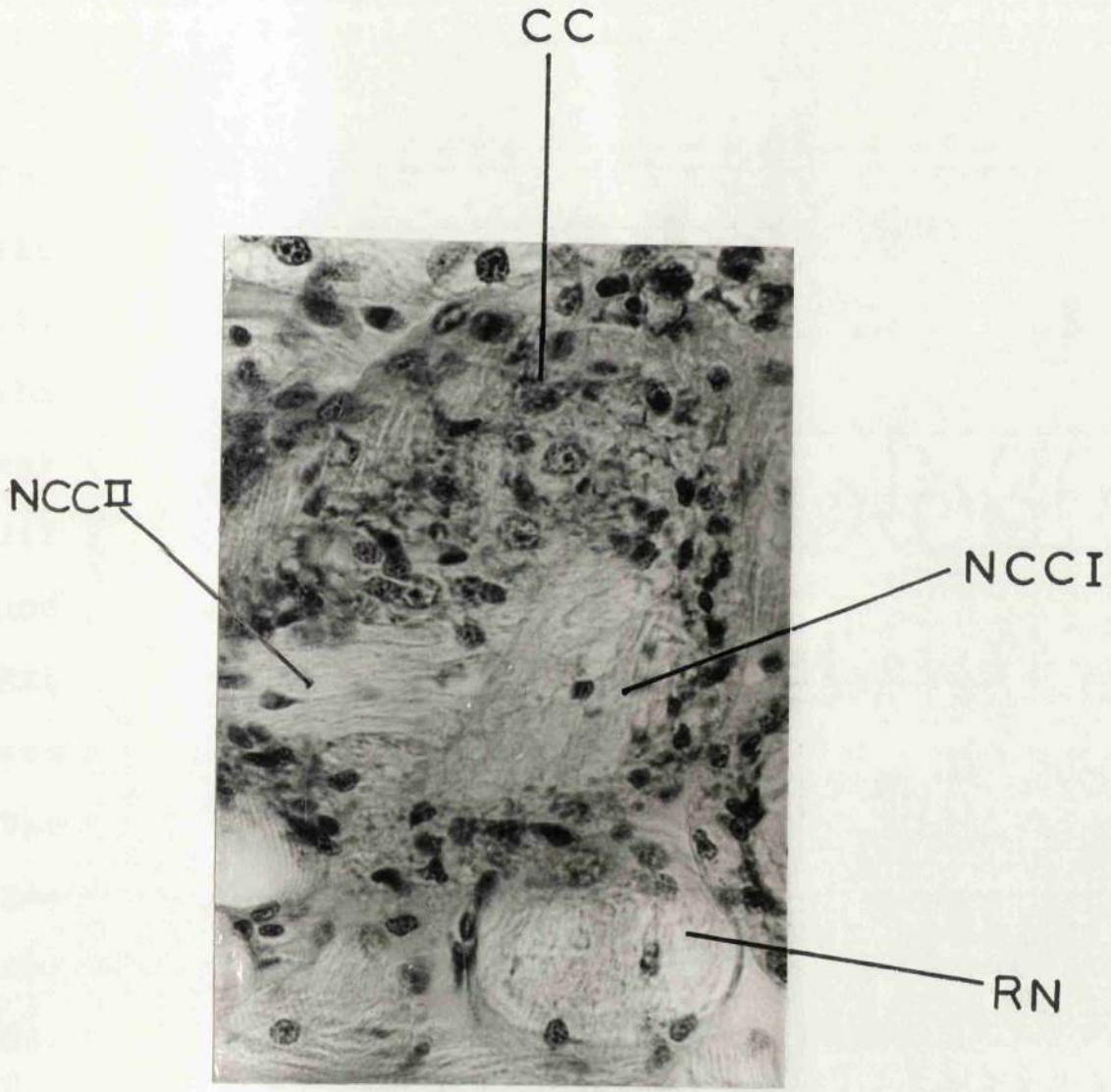


Figure 10.

neurosecretory cells lying in the pars lateralis beyond the corpora pedunculata. Each is thinner than its neighbouring NCCI and, unlike the latter, it does not cross over its partner of the opposite side in the brain (see Chapter 3). After making a direct exit from the lateral side of the protocerebrum, each NCCII lies lateral to its adjacent NCCI and at an angle to it. The presence of trachea and muscles and the fact that the NCCII is not a very thick nerve may sometimes make the tracing of this nerve as far as the CC difficult. However, the NCCII as well as the NCCI undoubtedly penetrate the anterior limit of the CC. Extensive crossing of fibres of the NCCI and NCCII seems to take place just after their penetration of these organs (Fig. 10). These nerves branch and re-branch in the peripheral and also in the central region of the CC and it becomes difficult to distinguish which axons belong to the NCCI and which to the NCCII.

IV(b)3. NCCIII. A pair of nerves originating in the two tritocerebral lobes and innervating the corpora cardiaca are known in Cicadidae (Pflugfelder, 1937), Phasmatodea (Nyst, 1942), Carausius (Dupont-Raabe, 1956) and P. americana (Drescher, 1960; Willey, 1961).

Figure 11. Photomicrograph showing the NCCIII and its two branches NCCIII:Br1 and Br2. Note also the corpus cardiacum (CC).

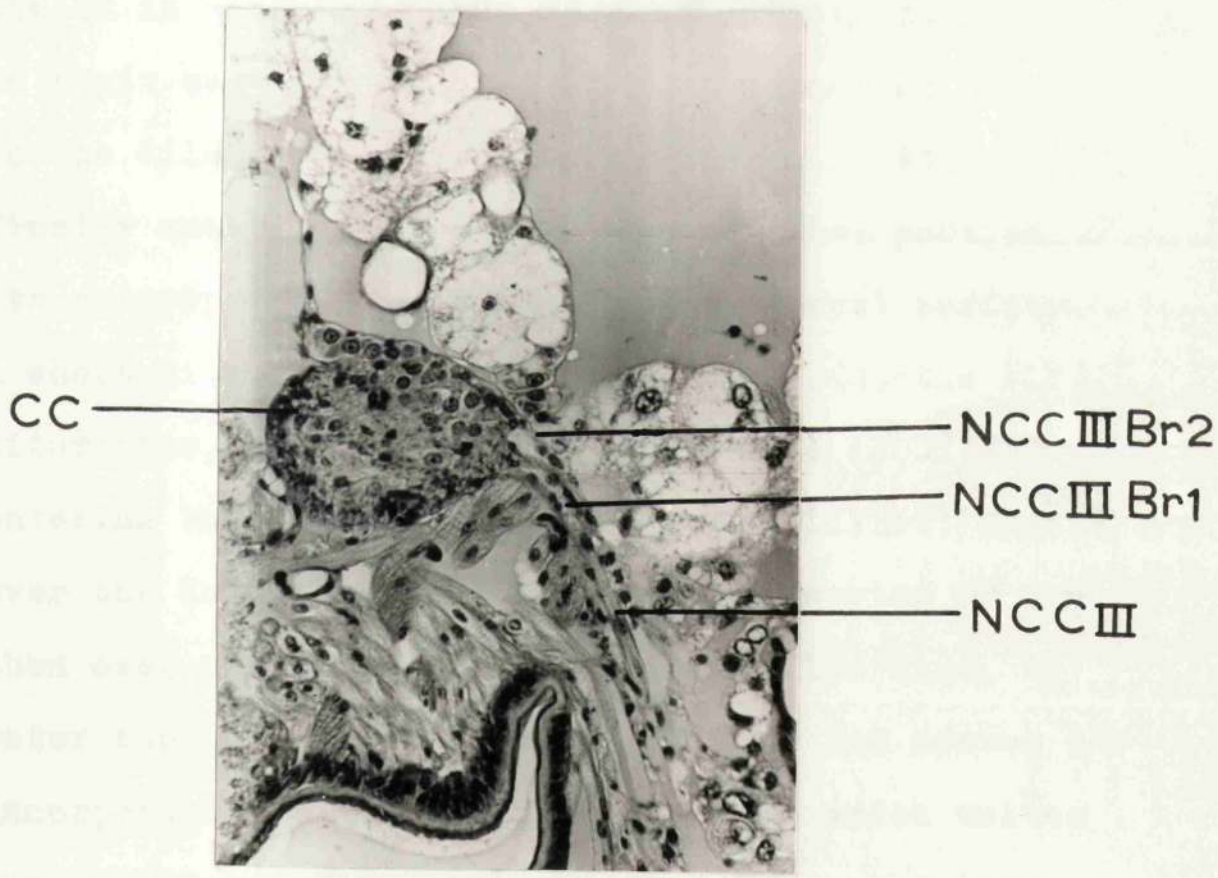


Figure 11.

These were named by Dupont-Raabe as the NCCIII and they have now been located in B. orientalis also.

The NCCIII are about the same thickness as the NCCII. The NCCIII arise from the anterior inner lateral surfaces of the tritocerebrum and run up towards the CC in a zigzag path, passing between muscle blocks on their way, and giving off nerve branches which go to the dilator muscles of the pharynx. Each NCCIII finally enters the anterior intra-aortal portion of its corresponding CC at its dorso-lateral surface. A short distance before it enters the CC, the NCCIII bifurcates, one bundle of nerve fibres (NCCIII:Br1) entering the CC while the other (NCCIII:Br2) passes over the dorsal surface of its neighbouring CC and then over that of the CC of the opposite side, to enter the latter (Fig. 11). The two Br2 nerves are incorporated in the dorsal commissure which unites the two CC and forms a landmark between the intra-aortal and extra-aortal portion of the CC. The axons of the NCCIII branch and re-branch in the CC. The cellular elements giving rise to NCCIII have not been located so far. The NCCIII occur in P. americana and B. orientalis. They have also been observed in

late embryos of P. americana (see Chapter 2).

IV(b)4. Structure of the corpora cardiaca. The anterior parts of the two CC are separate but are in contact with the dorsal aorta which is attached to the dorsal surfaces of the CC. The lumen of the aorta is wide at this level. Subsequently, the CC converge until they are in contact with each other but the boundary of each CC remains distinct. The anterior parts of the two CC which are incorporated in the ventro-lateral walls of the aorta (Figs 12 and 13), may have convolu-:ted surfaces. The posterior parts of the CC are not in direct contact with the dorsal aorta but are linked to it and to each other by the dorsal commissure. These extra aortal parts of the glands have relatively smooth surfaces. There are fewer cells in the extra-aortal part of a CC than in the intra-aortal part. Both parts are richly supplied with the endings of the three NCC.

The pericardial cells lie closely applied to the dorsal and dorso-lateral parts of the aorta. The lower lateral ends of the pericardial cells are in contact with the lateral part of the aorta which is in its turn in direct contact with the anterior parts of

Figure 12. Photomicrograph of a section through the anterior region (intra-aortal part) of the corpora cardiaca (CC) of a freshly moulted nymph of B. orientalis (chrome haematoxylin phloxin staining). Note numerous peripheral CH^+ granules, a few such granules are also present in the central part of the CC. RN, recurrent nerve; Ao, aorta; NCCIII should also be noted.

Figure 13. Photomicrograph of a section through the anterior region (intra-aortal part) of the corpora cardiaca (CC) of an adult male, P. americana, killed one hour after last moult. Lettering as in Figure 12.

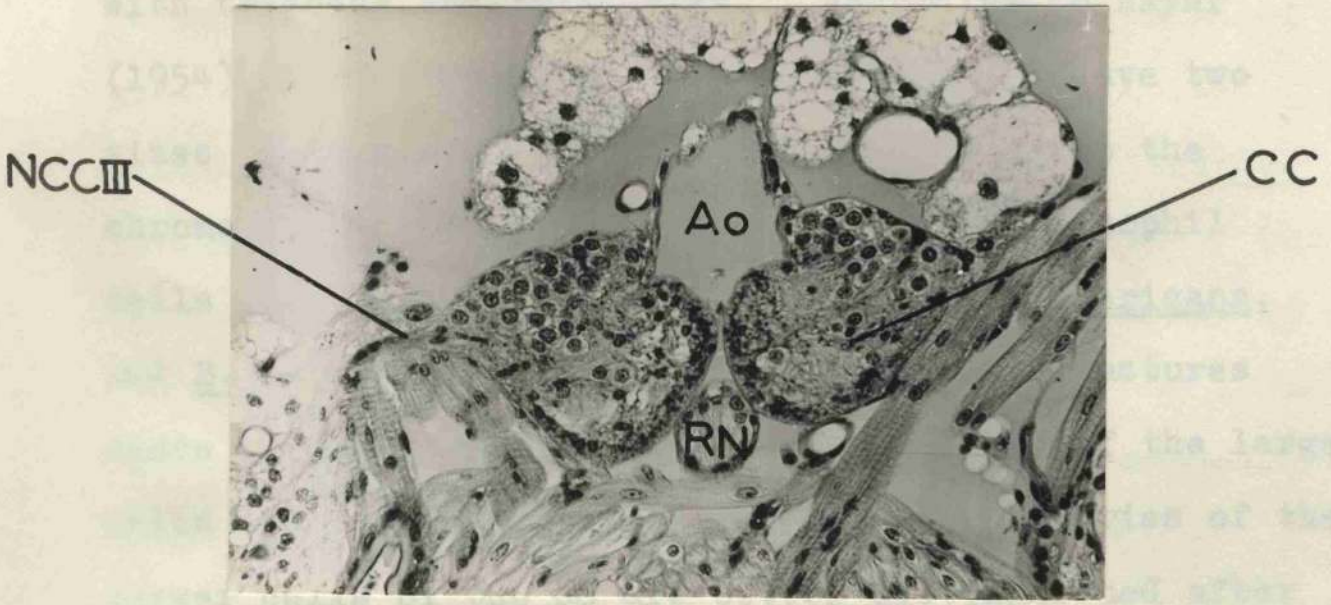


Figure 12.

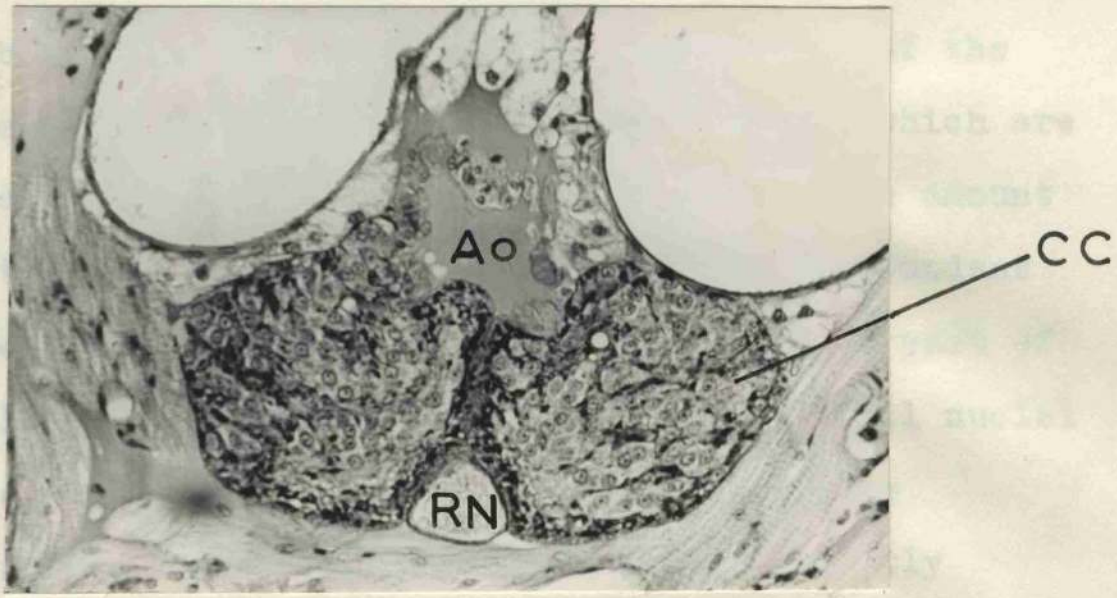


Figure 13.

the CC as mentioned above. The CC are richly supplied with tracheae and tracheoles. According to Nayar (1954) the CC of Locusta are syncytial and have two sizes of nuclei, the smaller corresponding to the chromophobe cells and the larger to the chromophil cells of Cazal (1948). But the CC of P. americana, and B. orientalis are not true syncytial structures since the cell boundaries of at least some of the larger cells are clearly visible. The cell boundaries of the larger cells of the CC are better distinguished after chromealum-haematoxylin-phloxin (CHP) staining than after the paraldehyde fuchsin-Groat's haematoxylin indigo carmine (PF) staining procedure. The fine granular cytoplasm of these cells of the CC stains pink after CHP staining, the chromatin of the nuclei blue-black and the nucleoli the intense red of the counterstain phloxin. These larger cells, which are characterised by the possession of a moderate amount of cytoplasm and a large nucleus, are more abundant in the intra-aortal than in the extra-aortal part of the gland. At least four other types of cell nuclei can be recognised in the CC: (a) the smaller chromophobe cell nuclei which are only slightly smaller than those of the chromophil cells and which have more chromatin than the chromophil cell nuclei

and are usually regarded as glia nuclei. This seems to be improbable since smaller more intensely stained glia nuclei can also be identified which are quite distinct from chromophobe cell nuclei; (b) elongated peripheral perineurium cell nuclei; (c) nuclei of the glia cells lying in between the other cells of the CC and also associated with the nerve fibres of NCC within the CC; and (d) the thin elongated tracheal nuclei associated with tracheae and tracheoles.

It is still not established whether the large CC cells are innervated by the axons of the NCC or not (Van der Kloot, 1960). However, a close examination reveals the presence at the periphery of some of the large cells of the CC of thin structures which assume the same colouration as neurosecretory material after appropriate staining. These structures resemble the intercellular nerve endings of the NCC containing neurosecretory material in other parts of the CC and may therefore be the terminations of axons of the NCC innervating at least some of the larger cells of the CC but it is equally possible that they are fine tracheoles since, unfortunately, tracheoles may also stain with CHP and PF stains. Thus, unless we discover a staining method which stains only the neurosecretory material and not the trachea or tracheoles

we cannot say with absolute certainty whether the CC cells are being innervated by the axons of the NCC or not. Engelmann (1957) has reported that the ventral surfaces of the posterior regions of the two CC are linked by nerve fibres but this does not occur in the two species considered here. The posterior part of each CC eventually bifurcates. The outer half, which contains abundant nerve fibres forms the nervus corporis allatum I (NCAI of Willey, 1961) and the inner half, which also contains nerve fibres, becomes the nervus cardiostomatogastricus (NCS) of Weber (1952) and Willey. According to Willey the NCS enter the HG separately but in fact they run backwards to unite with the RN, just before it enters the HG. Prior to the point of junction each NCS sends axons across the dorsal surface of the RN to its partner and so a transverse commissure is formed. After receiving the extra nerve fibres the RN becomes much broader and looks in transverse sections (Fig. 14), like a mushroom with a central stem containing its own original nerve fibres (coming from the frontal ganglion) and the dorsal and expanded lateral parts containing mostly fibres from the NCS. Posteriorly the stem disappears, and the nerve fibres of the RN and the NCS become intermingled and the classical

Figure 14. Photomicrograph indicating the recurrent nerve fibres (RN) and the nerve fibres of nervicardiostomatogatrici (NCS) B. orientalis nymph freshly moulted, chrome-:haematoxylin phloxin stain. Note the peripheral granules in the NCS.

Figure 15. Photomicrograph showing the hypocerebral ganglion (HG), nervi corporis allati I (NCAI), and the anterior part of the corpus allatum (CA) which has appeared in this section. Note the granules at the periphery of the hypocerebral ganglion.

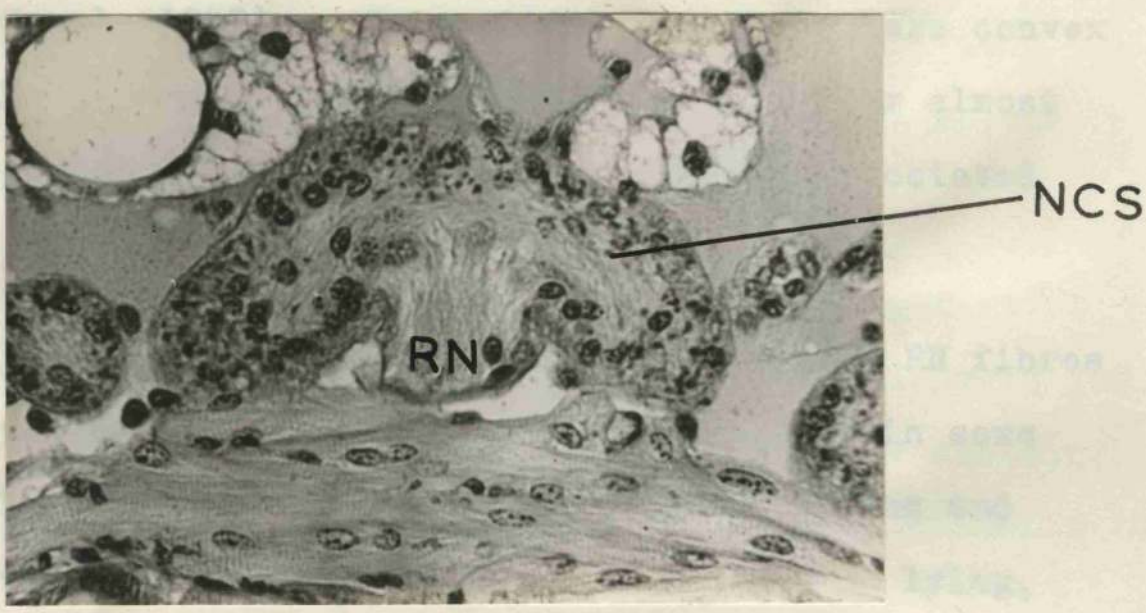


Figure 14.

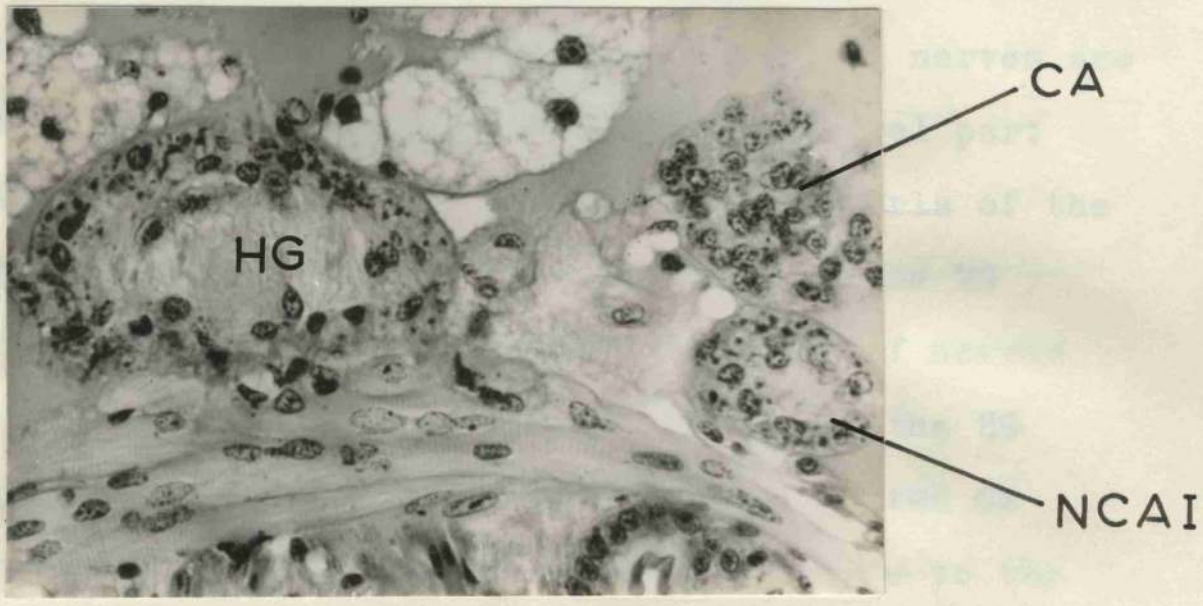


Figure 15.

hypocerebral ganglion is formed (Fig. 15), a broad structure lying above the gut and between the two CA (Cazal, 1948). In sections the HG appears convex on the dorsal surface and slightly convex or almost flat, on its ventral surface. Closely associated with it, ventrally, are one or two tracheae.

Those axons which intermingle with the RN fibres do not contain neurosecretory material but in some specimens, especially in newly moulted nymphs and adults, neurosecretory material can be seen lying, peripherally, on the dorsal, lateral and even ventral parts of the HG. This neurosecretory material actually occurs in axons contributed to the peripheral parts of the HG by the NCS. In the central part of the HG there are a few neurons and glia cells which are scattered among the nerve fibres. Several nerves are given off from the ventral and ventro-lateral part of the HG. These go to the tunica muscularis of the pharynx. A fine pair of nerves connects the HG with the NCAI, and another very fine pair of nerves arising from the ventro-lateral surface of the HG innervates the commissure which unites the two CA together. The dorsal aorta lies very close to the dorsal surface of the HG but posteriorly the former

separates from the latter. Although no definite nerve from the HG to the aorta could be seen, in a few sections when the HG and the dorsal aorta are in contact, a few fine nerve fibres from the HG appear to innervate the ventral wall of the aorta.

Finally, the HG becomes narrow at its posterior end and should now be called the oesophageal nerve (ON). At its anterior end the ON also occasionally contains peripheral neurosecretory material in axons contributed by the NCS.

IV(b)5. The dorsal commissure of the corpora cardiaca. (Figures 16-17). The dorsal commissure of the CC contains nerve fibres from the NCCIII. In the posterior region this commissure becomes thicker and is closely applied to the dorsal surface of the CC, separating the posterior region of the CC from the aorta. It is in intimate contact with the lateral and ventral wall of the aorta, the dorsal commissure forming the latero-ventral part of the aorta. Contrary to a recent assertion by Willey (1961) the dorsal commissure of the CC of P. americana (and that of B. orientalis is not asymmetrical. From the lateral side of the commissure several nerve fibres emerge to innervate the dilator muscles of the pharynx. Some

Figure 16. Photomicrograph showing the dorsal commissure (DC), posterior part of the corpora cardiaca (CC), recurrent nerve (RN), and the oesophagus (OES). Note that the ventral part of the dorsal aorta (Ao) is in close contact with the dorsal commissure of the corpora cardiaca. P. americana embryo 42-day-old, paraldehyde fuchsin, Groat's haematoxylin and indigo carmine stain.

Figure 17. Photomicrograph showing the dorsal commissure which has already separated from the dorsal aorta. Note that the dorsal commissure is symmetrical. B. orientalis nymph freshly moulted, chrome haematoxylin phloxin stain.

Lettering as in Figure 16.

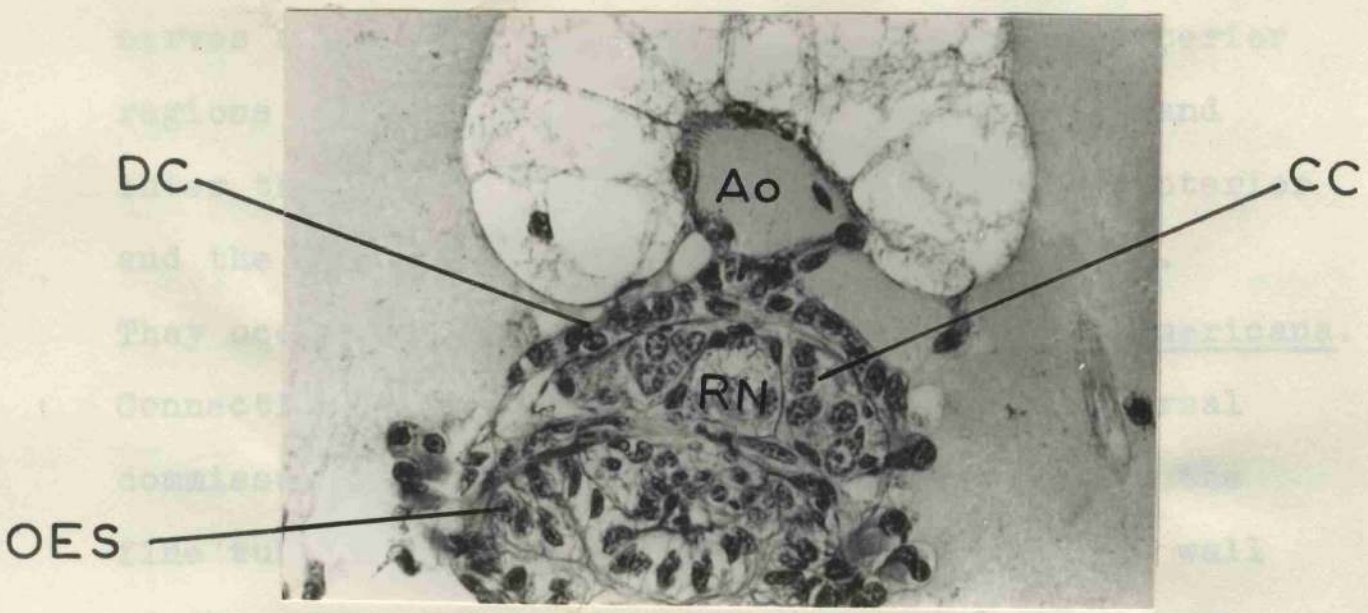


Figure 16.

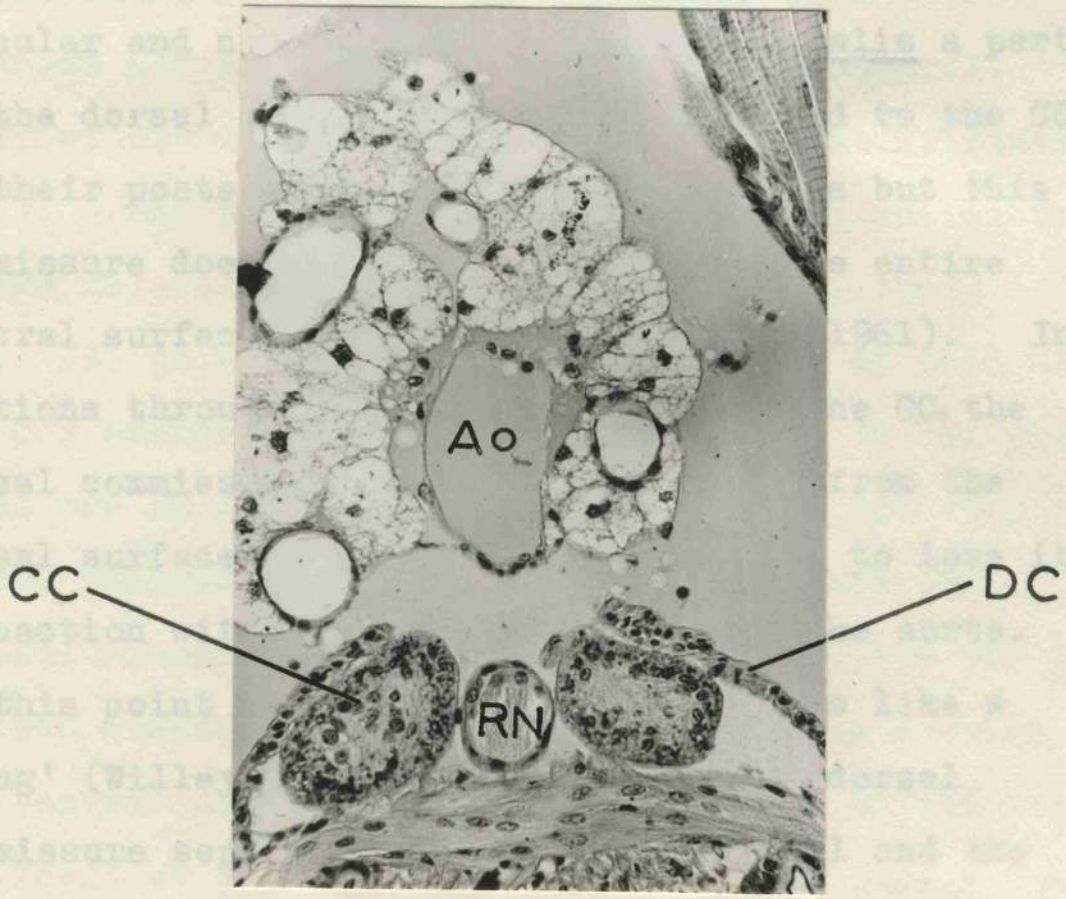


Figure 17.

of these fibres also innervate the tunica muscularis of the pharynx. An anterior and a posterior pair of nerves are given off from the anterior and posterior regions of the dorsal commissure respectively and enter the wall of the aorta. These are the anterior and the posterior nervi aortici (Willey, 1961). They occur in B. orientalis as well as in P. americana. Connection between the dorsal aorta and the dorsal commissure of the CC is further established by the fine tubular branches arising from the ventral wall of the aorta and penetrating the dorsal commissure lying just below the aorta. These connections, described here for the first time, appear to be muscular and not nervous. In B. orientalis a part of the dorsal commissure is also attached to the CC at their posterior ventro-lateral surface but this commissure does not extend throughout the entire ventral surface as in Blaberus (Willey, 1961). In sections through the posterior part of the CC, the dorsal commissure is slightly separated from the dorsal surface of the CC and also begins to lose its connection with the ventral surface of the aorta. At this point the dorsal commissure looks like a 'wing' (Willey, 1961). Eventually the dorsal commissure separates from the aortal wall and the

lateral part of the aorta is connected with two large lateral tracheae only. Two other large tracheae, lying above the aorta, were also noted. In posterior sections the dorsal commissure eventually disappears and the two CC are now no longer connected to each other. Nerve fibres, glia cells and haemocytes were observed in the dorsal commissure.

IV(c) Nervous connections and structure of the corpora allata

IV(c)1. Nervous connections. The nervi corporis allati I (NCAI) arise from the posterior ends of the CC and enter the CA at their anterior end. The axons of these nerves branch repeatedly inside the CA, intercellularly and thereafter they become difficult to trace. The two CA are themselves connected together by the allatal commissure (CCA of Willey, 1961) (Fig. 18) to which axons are contributed by both CA. The CCA emerges out from that portion of the CA which faces the HG and lies just above the gut and very close to the ventral surface of the HG. A close and careful examination reveals the fact that the CCA has its own sheath and though it lies in close contact with the ventral surface of the HG, it is not united with it except at two points where the CCA is joined to

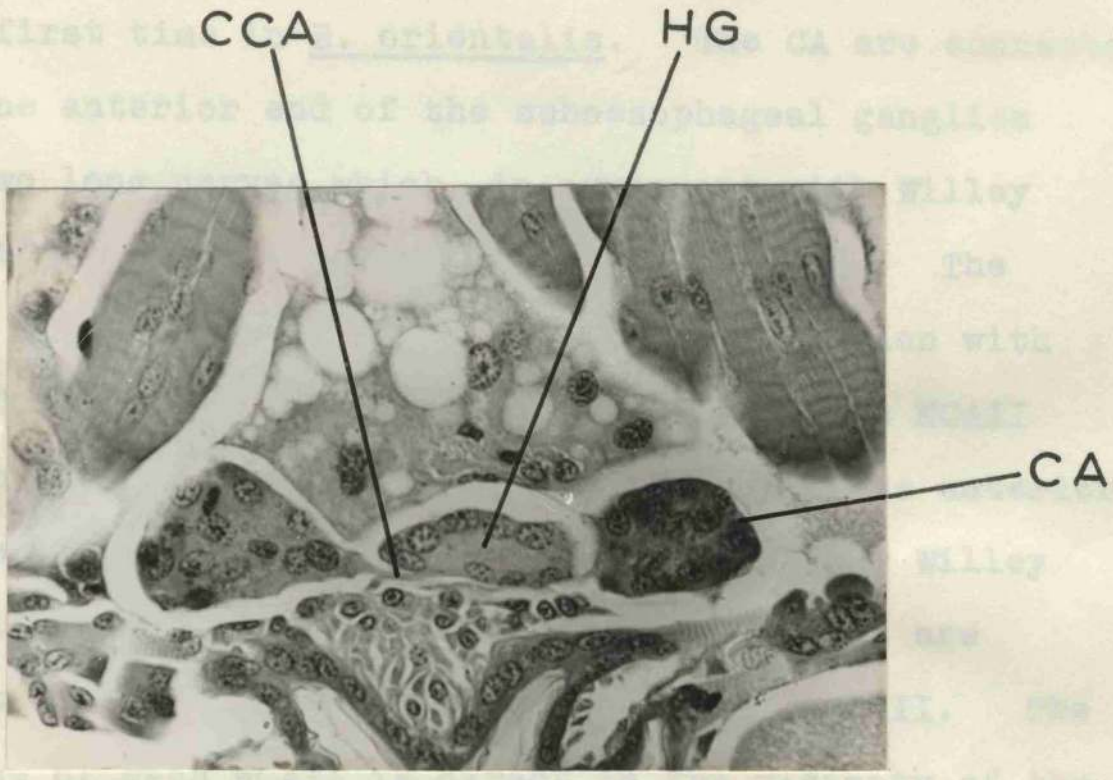


Figure 18.

the HG by a pair of fine nerves. The allatal commissure occurs in both species of cockroaches studied. Its presence in P. americana has been confirmed by Willey (1961) and is here reported for the first time in B. orientalis. The CA are connected to the anterior end of the suboesophageal ganglion by two long nerves which, in agreement with Willey (1961), will be called the NCAII (Fig. 19). The NCAI and NCAII meet at their point of junction with the CA. Prior to junction with the CA each NCAII gives off a branch which probably goes to the anterior end of the prothoracic glands, as claimed by Willey (1961). Haemocytes, as well as glia cells, are sometimes found between the axons of the NCAII. The course of each NCAII is direct in the vicinity of the CA but posterior to the CA it takes an indirect course to avoid muscle strands in its path. It is probable that NCAII receives nerve fibres from the posterior part of the CC via the NCAI (Harker, 1960) as well as from the suboesophageal ganglion, the latter source being reported by Engelmann (1957), and Willey (1961). The NCAII branch and re-branch inside the CA. A thin bundle of axons may be seen right in the centre of the CA. These emerge from the CA to form the allatal

Figure 19. Photomicrograph of a section through the corpus allatum (CA) of newly moulted nymph of B. orientalis showing nervus corporis allatum II (NCAII). Note also the hypocerebral ganglion (HG). Chrome haematoxylin phloxin stain.

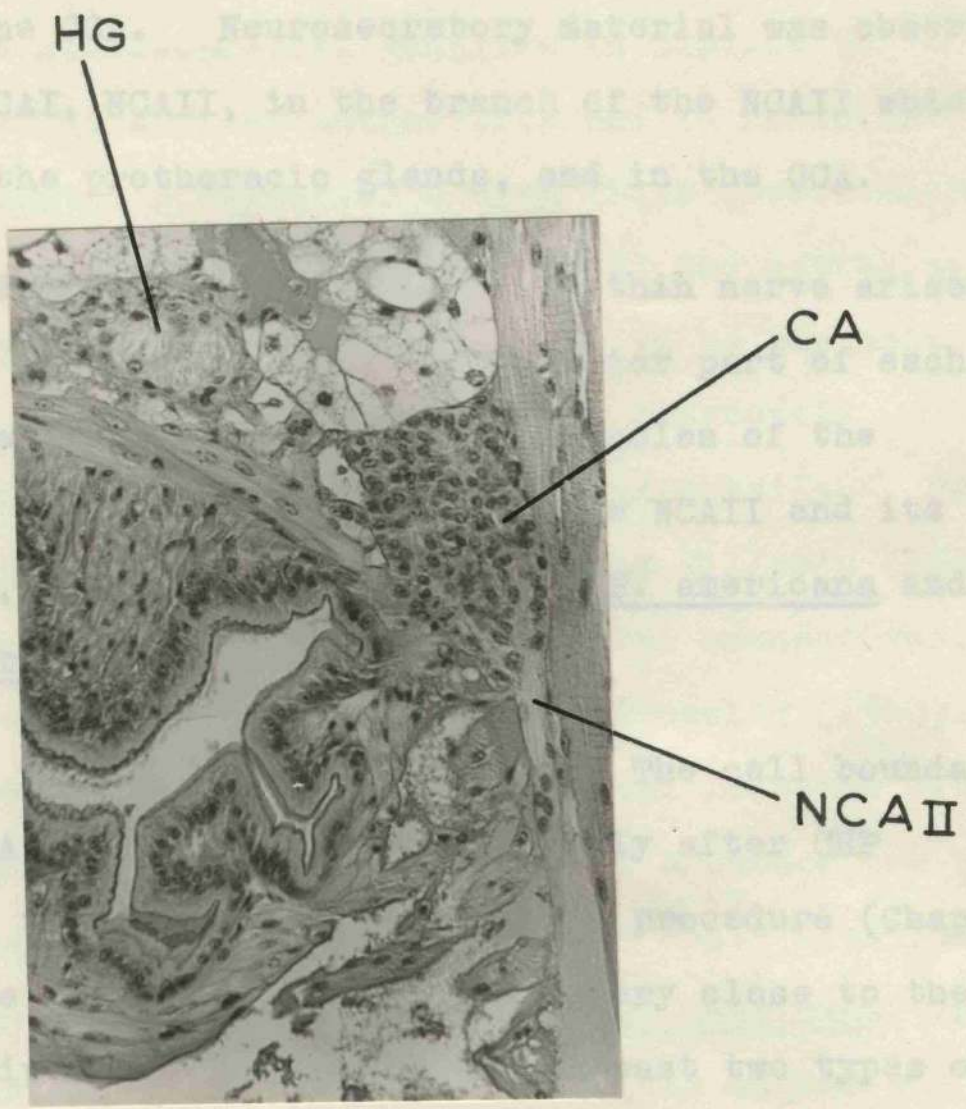


Figure 19.

commissure described above. It seems probable that fibres of the NCAI and NCAII cross over to the opposite CA via the CCA. Neurosecretory material was observed in the NCAI, NCAII, in the branch of the NCAII which goes to the prothoracic glands, and in the CCA.

Besides these nerves, another thin nerve arises from the ventral side of the posterior part of each CA and becomes lost in the outer muscles of the pharynx. The two CA, the NCAI, the NCAII and its branches, are asymmetrical both in P. americana and B. orientalis.

IV(c)2. The structure of the CA. The cell boundaries of the CA can be observed more easily after CHP staining than after the PF staining procedure (Chapter 3). The outer cells of a CA are very close to the thin periphery of the gland. At least two types of cells occur, namely smaller cells with small amounts of cytoplasm and small round nuclei, and larger cells with relatively large amounts of cytoplasm and larger nuclei. Phloxinophilic nucleoli are common in both types of cell nuclei whereas in those of the CC cells these are rare. Interaxonal glia cells and peripheral perineurium cells can also be distinguished.

DISCUSSION

The foregoing account of the microanatomy of the brain and suboesophageal ganglion is similar to that given by earlier authorities and it contains no fundamental disagreements with their observations. On the other hand, the description of the nerves of frontal ganglion, which is believed to be the most detailed yet produced, differs from previously published observations in a number of details. For nerve pair N_1 the author has accepted Willey's (1961), name 'connectivi frontales' or frontal connectives. These are the 'nervi frontales' of Drescher (1960). All three are agreed on the origin and location of the nervus connectivus, N_2. N_3 and its branches are not mentioned by Drescher, but these are dealt with fully and accurately by Willey (1961) in P. americana, and his description agrees with that of the author who has confirmed that the branches N_3A and N_3PI innervate the lateral retractor muscles of the labrum and that branch N_3PII innervates the precerebral dilator muscles of the pharynx. N_4 which innervates the tunica muscularis of the pharynx is described by the author and by Willey but is not mentioned by Drescher (1960). These are very fine nerves which can easily be missed in dissections but can be traced more readily in serial

sections.

Again the author and Willey are in agreement that the former's N_5 innervates the buccal lining and sends branches to the labroclypeal nerve. Drescher is mistaken in describing this simply as a labral motor nerve.

Finally, N_6 which arises from the ventro-lateral side of the frontal ganglion is described by Willey as coming from the posterior part of the ganglion and figured by Drescher as coming from the mid-lateral side of the ganglion. Willey says that this nerve appears to innervate the tunica muscularis of the pharynx but the writer, who was the first to trace the branches of this nerve (in both dissected specimens and histological preparations), is certain that these innervate the precerebral dilator muscles of the pharynx. The arrangement of the nerves of the frontal ganglion is essentially the same in B. orientalis as in P. americana.

The precise location of the HG has in the past caused some difficulty. Cazal (1948) said that the HG in B. orientalis, is a distinct swollen structure situated between the RN and the oesophageal nerve and lying between the two CA and containing a few scattered nerve cells. He further noted that the RN also

contains a few scattered nerve cells although he did not note the fact, that there are more nerve cells in the HG than in the RN. This description of the position of the HG agrees with that of Nesbitt (1941) who saw this ganglion in Blaberus as a broad flat structure behind the CC. Drescher (1960) ignored the existence of the HG but Willey (1961) said that in P. americana, it is represented by a diffuse linear arrangement of small nerve cells in the RN extending from the level of the anterior dorsal commissure of the CC to a point beyond the commissure of the CA. In none of his figures has he shown the HG as a really distinct localised swelling. The ganglion as described by Willey lies anterior and posterior to the junction of the nervi cardiostomato-gastrici. As an added complication he labels structures, in his figures 18 to 22, as the RN which according to his description belong to the HG.

Since nerve cells occur intermittently throughout the entire length of the RN it is obvious that these cannot be used as markers of the HG. It therefore seems sensible to consider that this ganglion is formed by the junction of the RN and NCS. Beyond this point it is a broad definite structure in both species of

cockroaches as described by Nesbitt, and Cazal, and as figured by Füller (1960), and Harker (1960) in P. americana.

After giving off nerves to the tunica muscularis of the pharynx, to the NCAI, and to the commissure of the CA, the HG narrows just behind the posterior limit of the CA and at this point it gives off the oesophageal nerve. This point of origin of the oesophageal nerve may be considered as the posterior limit of the HG.

Regarding the nervous connections of the CC three branches of NCCI have been recorded here for the first time. Contrary to the accepted definitions of NCCII, as nerves formed by the axons of lateral neurosecretory cells, Willey has suggested that the NCCII of P. americana have two roots, the one in posterior median neurosecretory cells of the pars intercerebralis (his group 4) and the other in neurosecretory cells lying 'just posterior and ventral to the posterior calyx of the corpus pedunculatum'. He has since made it clear that the latter are located in the pars lateralis (personal communication). The axons of the neuroscretory cells in the posterior median location (see Chapter 4) do not in fact join with those of the lateral neurosecretory cells in either P. americana or B. orientalis, and the idea of a dual origin of the

NCCII must be rejected.

According to Willey (1961) the dorsal commissure of the CC of P. americana are highly asymmetrical and he assumes that such asymmetry in this structure is characteristic of cockroaches, but this is not so in that species or in B. orientalis. It is clear from the photomicrograph used by Willey to illustrate this point that the section was oblique. However, Willey is correct in describing the CA, NCAI, NCAII and its branch as asymmetrical.

Willey claims that the CC of Blaberus have a convoluted surface 'when viewed in histological preparations' while those of Periplaneta possess a relatively smooth surface. Unfortunately, his figure 18 represents a section through the posterior or extra-aortal part of the CC of Periplaneta while his figure 19 shows a section through the anterior or intra-aortal part of a CC of Blaberus. So the two sections which he selects to illustrate his points are not comparable. The anterior intra-aortal portions of the CC of both P. americana and B. orientalis are in fact convoluted or grooved to a degree which varies from individual to individual and depends on the amount of neurosecretory material present in the gland at a given time. In contrast the posterior extra-aortal part is usually

smooth. Although the author has not studied actual sections of the CC of Blaberus he has compared Willey's figure 19 with a section of the same region of the CC of Periplaneta and has found no significant difference in the degree of convolution of the CC of these two species.

Although the question of neurosecretion is dealt with fully in later chapters, the question of the occurrence of neurosecretory material in the HG and oesophageal nerve must be dealt with now since it involves the interpretation of anatomical details. The occurrence of peripheral neurosecretory granules in the HG and in the proximal part of the oesophageal nerve of cockroaches was reported by Füller (1960). He was unable to explain why this neurosecretory material did not occur all along the oesophageal nerve. It has now been established, in both species investigated, that while some axons of the nervi cardiostomatogastrici lacking secretory granules intermingle with the axons of the RN in the neuropile of the HG, other axons of the nervi cardiostomatogastrici, containing neurosecretory material do not. The latter terminate close to the surface of the HG so that in sections of the posterior half of the HG abundant neurosecretory granules can be

found, at certain times, e.g. in freshly moulted nymphs and adults, at the periphery of the HG. These NCS (which really belong to the neurosecretory cells of the protocerebrum) do not all terminate in the HG some extending beyond the posterior limit of the HG to terminate close to the dorso-lateral surfaces of the oesophageal nerve. The last mentioned terminations account for the presence of neurosecretory material in the proximal part of the oesophageal nerve.

CHAPTER 2

EMBRYONIC DEVELOPMENT OF BRAIN OF PERIPLANETA AMERICANA L.

INTRODUCTION

Although the embryonic development of orthopteran (old classification) insects has been extensively studied, the development of the brain and brain centres has not received a great deal of attention. Most of the work on the development of the brain has been incidental to other embryological studies (e.g. Wheeler, 1893; Heymons, 1895 and Roonwal, 1936, 1937).

The large actively-dividing cells in the embryonic brain are commonly referred to as 'neuroblasts', a term originally used by Whitman (1878, 1887) to designate the two cells derived from the posterior macromere of Clepsine eggs which gave rise by process of budding to two rows of cells, the neural rows from which the nerve-cord is ultimately formed. His (1889), in turn used the term 'neuroblast' for the products of the 'Keimzellen' which gave rise to ganglion cells, but Wheeler (1893) pointed out that His should have used the term for the Keimzellen and not for their products.

Wheeler (1893) in his work on the neurogenesis of

Xiphidium, described two types of cells in the developing central nervous system, large pale cells with spherical nuclei and, lying above these, smaller darkly stained cells with oval nuclei. The former he called neuro-:blasts because they give rise to the central nervous system. He designated the latter dermatoblasts because they give rise to the dermatogenic cells which go to form integumental structures.

Neuroblasts and dermatoblasts are both ectodermal in origin. Since then, neuroblasts and dermatoblasts have been recognised in many insect species. In 1891 Wheeler stated correctly that the small cells produced by unequal division of neuroblasts divide to form ganglion cells although in 1893 he revised his opinion and stated that the immediate products of the neuro-:blasts are directly transformed into ganglion cells without further division. Since then some investigators, notably Bauer (1904), Nelson (1915), Poulson (1950) and Panov (1957) have supported Wheeler's (1891) earlier opinion while others, notably Eastham (1930), Baden (1937) and Johannsen & Butt (1941) have supported Wheeler's (1893) later notion that the small cells budded off from neuroblasts undergo no further division but are directly converted into ganglion cells.

There has been a notable difference of opinion as to whether neuroblasts take part in the formation of the optic lobes as they do in the formation of other parts of the brain.

Viallanes (1890) first assumed that there were no neuroblasts in the optic lobe of Mantis but later he observed gangliogenes, i.e. neuroblasts, in the lamina ganglionaris of the optic lobe. However, he failed to see the neuroblasts in the intraganglionic thickening which were subsequently discovered by Bauer (1904). According to Wheeler (1893) the embryonic cells of the optic lobe of Xiphidium resemble the neuroblasts in their poor affinity for stains but differ from them in that they are elongate whereas neuroblasts are round. Heymons (1895) in his account of the development of Forficula said that there are large cells in the optic ganglion of the embryo which resemble neuroblasts, but which, in fact, are not; while Johannsen & Butt (1941) and Richards & Davies (1957) stated that the optic lobe of the orthopteran insects is formed from ectodermal cells by delamination and that neuroblasts do not take part in its formation. Finally, Roonwal (1937) and Baden (1937) believed that neuroblasts do not occur at any stage in the optic lobes of either Locusta or Melanoplus, respectively.

According to them the cells of the optic lobe which divide and form this part of the brain are different at all embryonic stages from neuroblasts, and this type of cell is not found at any time in any other part of the brain.

The intraganglionic thickening which lies between the first and second protocerebral lobes has been described in embryos of Mantis by Viallanes (1890), and in those of Xiphidium by Wheeler (1893). According to them it separates from the integument completely at a certain stage of embryonic development and forms a deeply staining mass between the first and second protocerebral lobes. They believed that it migrates deep into the brain, disappearing for a time but reappearing later to become lodged between the medulla externa and the medulla interna of each optic lobe. They also held that at a still later stage the intraganglionic thickening atrophies.

Baden (1937) did not agree with this supposed disappearance and reappearance of this structure. He also questioned the mechanism by which such a large structure could migrate deep into the brain and decided that the term intraganglionic thickening should not be applied to the hypodermal incurving between the

first and second protocerebral lobes of his 'stage seven' embryo of Melanoplus. He thought that this was simply a hypodermal indentation which disappears as the processes of cephalic fusion advance, and that it has no connection whatsoever with the structure which appears intraganglionically at his 'stage thirteen' deep in the brain between the medulla externa and the medulla interna. It fell to Bauer (1904) to state with certainty that the lamina ganglionaris and the intraganglionic thickening are the first and the second formation centres, respectively, of the optic ganglion of insects. Strindberg (1913) found neuroblasts at the base of the intraganglionic thickening as well as those within that formation centre described by Bauer, but Baden (1937) could not find these in Melanoplus. Strindberg also believed that there is a second intra-:ganglionic thickening between the second and third protocerebral lobes.

Regarding the neuropile masses of the optic lobe Roonwal (1937) briefly described the neuropile of the lamina ganglionaris, the medulla externa, the medulla interna, external and internal chiasmata, the post-retinal fibres and the optic nerve in the embryonic optic ganglion of Locusta and confirmed the earlier

work of Viallanes (1891) on Mantis, but Baden (1937), (who examined the embryological development of the central nervous system of Melanoplus only as far as the completion of blastokinesis, omitting the development of the association centres) cast doubt on the terms used by Viallanes such as medullary masses, optic nerve of the optic ganglion, etc.; and, failing to find either the external or the internal chiasmata or an internal medullary mass (medulla interna) doubted their very existence in the embryo. However, Bauer's (1904) observations also supported those of Viallanes.

There is again disagreement among the earlier workers about the origin of perineurium cells, the neural lamella, and glia cells. For example, six possible sources of perineurium cells have been suggested, three postulating an ectodermal origin and three a mesodermal origin. Heymons (1895) thought that the outer neurilemma, i.e. neural lamella plus perineurium, is derived in Forficula from those dermatogenic cells which are left in the periphery of the brain after the separation of the main mass of dermatogenic cells from the brain cells. But according to Strindberg (1913) this is definitely not true in Eutermes, Formica and Chrysomela, in which the

outer neurilemma is formed when the ganglion has already separated from the hypodermis and so the former could not possibly be derived from the latter. Nusbaum (1883), Wheeler (1893) and Tiegs & Murry (1938) all said that it arises from the intraganglionic sections of the median cord but Baden (1937) rejected this hypothesis. Two possibilities were suggested by Nelson (1915) who studied the development of Apis. The first was that the outer neurilemma was derived from the dermatoblasts or dermatogenic cells as suggested by Heymons (1895), and the second and, he thought, more probable was that outer ganglion cells were transformed into the cells of outer neurilemma.

Strindberg (1913), Eastham (1930), in Pieris, and Roonwal (1937) also held that the outer neurilemma arises from the outer ganglion cells which are the outermost products of the neuroblasts. The fourth possibility was suggested by Baden (1937) who reported in Melanoplus that "the external neurilemma can be traced to coelom sacs and therefore seems to be mesodermal". According to Pflugfelder (personal communication) the perineurium cells which form the inner layer of the outer neurilemma arise from the neural rudiments of the mesenchyme, that is to say that perineurium is ectomesenchymal in origin. Finally,

Korotneff (1885) held that the outer neurilemma arises from the wandering blood cells which enter the developing nervous system, thus implying like Baden (1937) and like Pflugfelder that it is mesodermal in origin.

All the earlier workers to whom reference has been made in this context have treated the neural lamella and the perineurium jointly as a single structure, the outer neurilemma, but more recently these have been considered separately and the question of the origin of the neural lamella has now arisen. Scharrer (1939), and Wigglesworth (1959a) have suggested that the neural lamella is secreted by the underlying perineurium cells, but Wigglesworth (1956) indicated the possibility that haemocytes also contribute to the formation of the neural lamella.

The neural lamella stains with dyes which stain the connective tissue of the vertebrates, one of the facts which led Scharrer (1939) to believe that the perineurium cells which produce it represent connective tissue elements (see below).

However, Pipa (1961a) quotes extensive evidence to show that the neural lamella resembles vertebrate basement membrane in its histological properties.

In the case of glia cells there is general support for the theory that they have an ectodermal origin. According to Strindberg (1913) the neuropile is fully covered by ganglion cells before the appearance of glia cells and he considered that the ganglion cells close to the neuropile subsequently differentiate to form glia cells. Pflugfelder (personal communication) holds the view that the glia cells and ganglion cells have, undoubtedly, a common origin but that they subse-:quently differentiate in different directions. Bauer (1903, 1904) thought that some glia cells in the developing central nervous system of the prepupa of Culex were recruited from the haemocoele, and were thus of mesodermal origin, although he did not exclude the possibility that other glia cells might be formed by the differentiation of ganglion cells. Haller (1905) rejected this since he considered that the immigrants were phagocytes. It was suggested by Scharrer (1939) that glia cells have a nutritive function. She regarded perineurium cells as connective tissue cells and she distinguished them from glia cells (which she regarded as true neuroglia or supporting cells) by the fact, (a) that they do not have processes extending between neurons as do glia cells, (b) that they do not possess either the fibrils or the gliosomes

which are characteristic of glia cells in cockroaches, and (c) that they have different supra vital staining characteristics. Wigglesworth (1959a, 1960) does not draw such a sharp distinction between perineurium cells and glia cells, regarding the former as specialised glia cells. He has shown that they have complementary functions. Assuming that the neural lamella is freely permeable, he stated that the perineurium cells regulate the passage of solutes into the ganglia and store glycogen whereas glia cells transfer glycogen and fat to ganglion cells through Holmgren's (1900) 'trophospongium'.

Pipa (1961a) has reserved his opinion as to whether the perineurium cells are connective tissue elements or neuroglia since conclusive embryological and comparative histological information on this subject is so scarce. However, he does contribute the observation that perineurium cells have a greater concentration of demonstrable phospholipids than the glia cells.

The transverse nervous connection between the two halves of the protocerebrum lying above the central body, is usually termed the supraoesophageal commissure (pons cerebralis) while the extra-cerebral nervous connection

between the tritocerebral lobes is called the suboesophageal or substomodaeal commissure. Both were thought by Heymons (1895) to arise, in Forficula, from a median ectodermal thickening. Strindberg (1913) held that, in Eutermes, the supraoesophageal commissure arises from the protocerebral lobes; and yet he derived the suboesophageal commissure from the median ectodermal thickening. Nelson (1915) maintained that in Apis the former arises from the median ectoderm; but he was uncertain about the origin of the latter. However, Roonwal (1937) did not consider that dermatogenic cells play any part in the formation of these commissures in Locusta, and he correctly stated that the former arises from the protocerebrum and the latter from the trito-:cerebrum, i.e. from the ganglia they unite.

A pair of cellular structures lying side by side and ventral to the stomodaeum, and hence called the suboesophageal bodies, have been observed in embryos of many insects. According to Wheeler (1893) and Roonwal (1937) these have a mesodermal origin in Xiphidium and Locusta respectively. Two possible functions have been ascribed to these. Wheeler (1893) compared them with crustacean green glands, and Heymons (1895) and Roonwal (1937) also thought that their function was excretory, but Toyama (1902) and

Pflugfelder (personal communication) have maintained that these produce blood cells in the embryo. Eastham (1930) in his study of Pieris embryo dismissed the idea of a leukopoietic function. Heymons (1895) claimed that they persist in nymphs of Gryllus and of Blattidae.

The origin and development of the corpora cardiaca (CC) have been studied by Wiesmann (1926) and Pflugfelder (1937, 1952) in Carausius, by Roonwal (1937) in Locusta and by Baden (1937) in Melanoplus. All agree that the CC develop as paired ectodermal outgrowths of the dorso-median part of the embryonic stomodaeum. The unpaired hypocerebral ganglion (HG) forms at the same time and, later, the CC separate from the HG and become connected with the cardioblasts of the developing aorta.

There is some disagreement about the origin of the corpora allata (CA). It was originally suggested by Wheeler (1893) that the CA originate from the mesoderm of the tritocerebral segment in Xiphidium, and Blatta. Strindberg (1913) also thought that the CA have a mesodermal origin in Eutermes, but that the CA have an ectodermal origin in Formica. All other authorities have agreed to an ectodermal origin, the observation of Heymons (1899) that they develop from ectodermal invaginations which form at the junction of the

mandibular and maxillary segments in Forficula being generally accepted although in Gryllus he found that they originate from a solid group of cells. Pflugfelder (1937, 1952), and Roonwal (1937) have pointed out that the timing of events in the development of an embryo may be such that a structure which is really an invagination may appear as a solid mass of cells, the space within developing later. This space, which represents the lumen of the invagination, is subsequently obliterated. Others who support the theory of ectodermal origin include Nusbaum & Fulinski (1906, 1909), Eastham (1930, in Pieris) and Nelson (1924, in Apis).

MATERIAL AND METHODS

Oothecae of Periplaneta americana L., were removed from females at the stage when three-quarters of the ootheca projects from the maternal body. The micro-:pyles of such oothecae had already closed. The age of an embryo was counted from the time an ootheca was removed from the body. These oothecae were kept in separate open-mouthed specimen tubes and the date and time of collection were noted on each tube. Eggs were incubated at 25°C (± 2°C), development taking 55-56 days at this temperature. An average of

fifteen first instar nymphs hatched out of each of the oothecae which were left to develop. Other oothecae were opened at various times in order to obtain a series of embryos at all stages of development. These oothecae were opened up in a 50:50 mixture of Ephrussi & Beadle's (1936) insect Ringer solution (7.5 gm. sodium chloride, 0.35 gm. potassium chloride, 0.21 gm. calcium chloride and 1,000 ml. distilled water) and aqueous Bouin, under a binocular microscope. The opened oothecae were left in this mixture for about an hour. This treatment prevents the rupture and disintegration of the eggs by making them hard and thus easier to dissect out in the early embryonic stages. The embryos become conspicuous after fixation. It is difficult to remove entire early embryos from eggs without damaging them so individual eggs were transferred, after removal of the chorion and the serosa only, to aqueous Bouin solution for 6 to 12 hours. Ten-day and older embryos were large enough to be dissected out of their eggs in the same saline-fixative mixture and were fixed in aqueous Bouin for 6 to 12 hours.

After dehydration, specimens were embedded in Steedman's (1947) ester wax. Frontal and sagittal sections were cut at 6μ and were stained by the

standard paraldehyde fuchsin procedure, counterstained with Groat's haematoxylin and indigo carmine (see Chapter 3).

OBSERVATIONS

Section I: Neuroblasts, Ganglion Mother Cells and Ganglion Cells (Figures 20, 21)

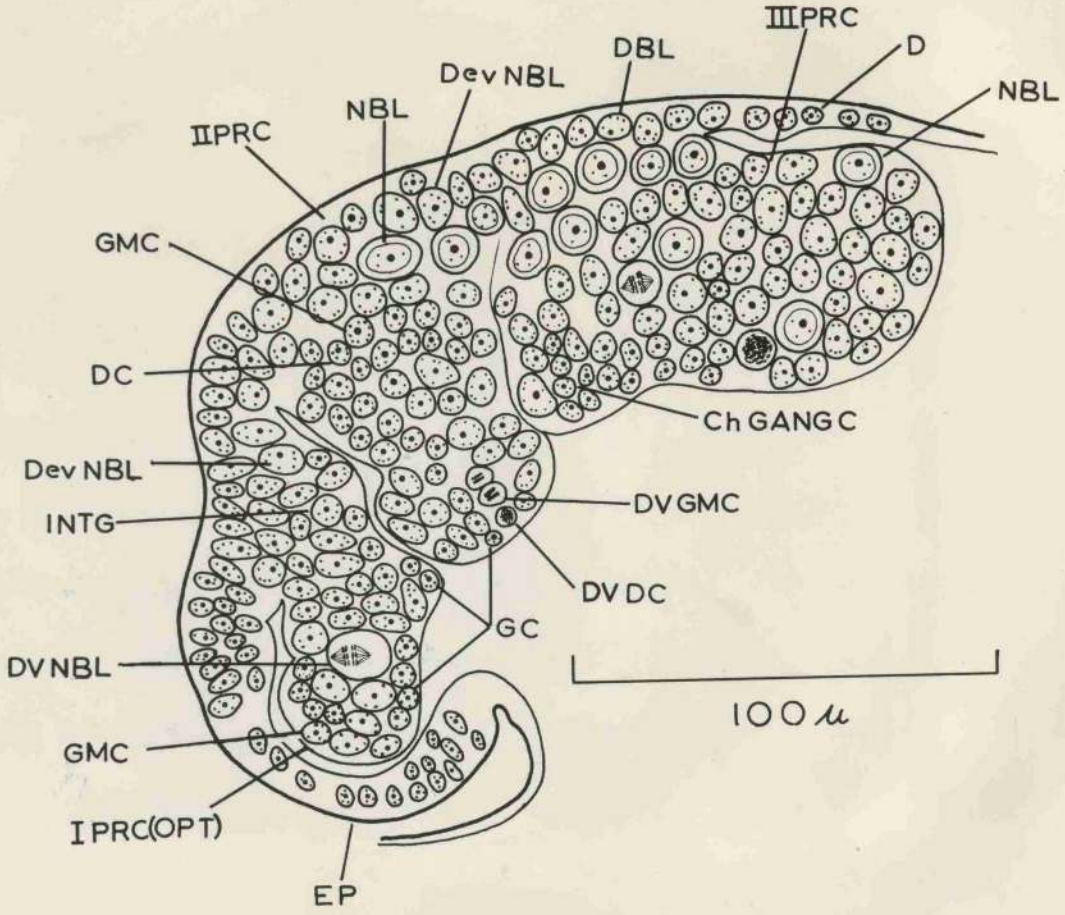
Neuroblasts were first seen as small clusters of cells in 5-day embryos. These flask-shaped differentiating neuroblasts occur in the embryonic brain of *P. americana* up to the tenth day of embryonic development. From the eleventh to the twenty-fifth day the neuroblasts are arranged in a continuous line along the periphery of the brain. Above these neuroblasts lie the dermatoblasts. The neuroblasts, which give rise to the central nervous system, and the dermatoblasts which go to form the integumental structures, are both ectodermal in origin.

Resting, and characteristically unequally dividing, neuroblasts of different sizes, together with ganglion mother cells (see below), were observed in the embryonic optic ganglion and indeed throughout the entire brain.

Mature neuroblasts of *P. americana*, as in other

Figure 20. Camera lucida drawing of a frontal section passing through the anterior part of a 10-day-old embryo of P. americana. I, II, III PRC, 1st, 2nd and 3rd protocerebral lobes; D, dermatogenic cell; DBL, dermatoblast; NBL, neuroblast, DVNBL, dividing neuroblast; GMC, ganglion mother cell; DVGMC, dividing ganglion mother cell; DC, daughter cell; DVDC, dividing daughter cell; GANGC, ganglion cell; ChGANGC, chain of ganglion cells; DevNBL, developing neuroblast; INTG, intraganglionic thickening; OPT, optic lobe; EP, eye plate. The nuclear boundary of mature neuroblasts have been shown only.

Figure 21. A composite camera lucida drawing from several frontal sections passing through the anterior part of a 10-day-old embryo of P. americana. Lettering as in Figure 20.


IIIPRC
D
DBL
Dev NBL
NBL
NBL
IIPRC
GMC
DC
Ch GANG C
Dev NBL
INTG
DV GMC
DV DC
GC
DV NBL
100 μ
GMC
IPRC(OPT)
EP

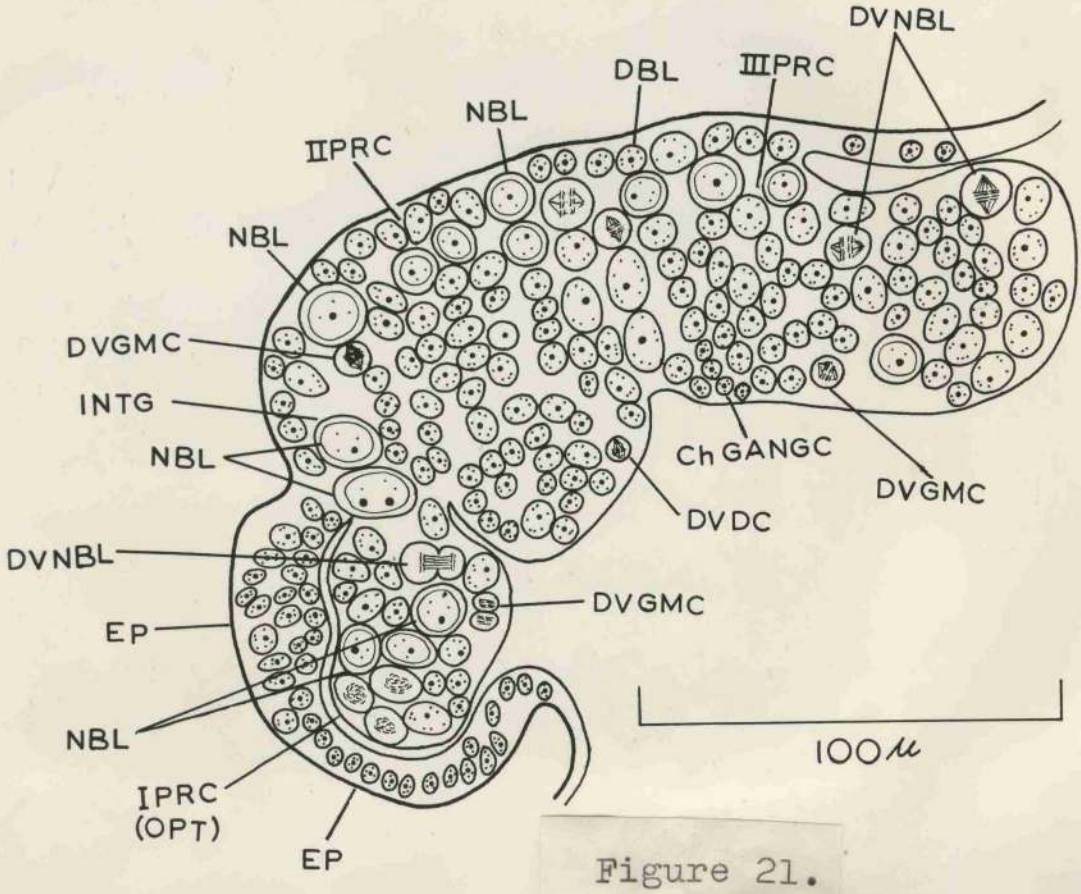

DV NBL
DBL
IIIPRC
NBL
IIPRC
NBL
DV GMC
INTG
NBL
Ch GANG C
DV GMC
DV DC
DV NBL
DV GMC
EP
NBL
100 μ
IPRC
(OPT)
EP

Figure 21.

insects, are round, or oval in section. These cells, which vary in size, have abundant cytoplasm but their nuclei appear to be poor in chromatin material, at least during interkinesis, i.e. the resting stage between first and second mitotic division. These nuclei sometimes possess two nucleoli during inter-:kinesis. Dermatoblasts and their products (formed by equal division), dermatogenic cells, are poorer in cytoplasm than neuroblasts, but their nuclei appear to be richer in chromatin material. Most of the large peripheral neuroblasts divide unequally and at right angles to the overlying dermatoblasts. The unequal division of neuroblasts first becomes noticeable during the late metaphase and is obvious during telophase. The smaller cells produced by this unequal division are ganglion mother cells or 'pre-ganglion cells' (Poulson, 1950) which in P. americana certainly divide equally to give rise to two daughter cells. Daughter cells may become directly differentiated as ganglion cells which ultimately develop into neurons but some ganglion cells are smaller than daughter cells and pairs of these which have been observed in metaphase and telophase are obviously derived by the division of daughter cells. The 'dividing ganglion cells'

reported by Baden (1937) in Melanoplus, and by Kawamura in a grasshopper, Chortophaga (personal communication), are probably dividing daughter cells. The neuroblasts, meanwhile, continue to divide unequally and gradually become smaller in size. How many times a given neuroblast divides is not known. It may be that in the last stage the small neuroblasts undergo equal division to give rise to two ganglion mother cells or directly to two ganglion cells but a few neuroblasts along the periphery of the brain certainly appear to be degenerating in the 20-day embryo and by the thirty-fifth day the number of peripheral neuroblasts has fallen drastically though a few persist into the later embryonic stages. Thus the ultimate disappearance of these cells in the late embryonic life may be due partly to the ultimate division of some to a ganglion cell end point and partly to the degeneration of others.

After 42 days of embryonic development mitotic activity of neuroblasts and ganglion mother cells is confined largely to the following three sites in each half of the brain:

(1) In the two formation centres of the globuli cells of the mushroom body (corpus pedunculatum)

Figure 22. Photomicrograph of a frontal section through the brain of a 42-day-old embryo, showing the formation centre (FC) of the globuli cells (GC). This formation centre lies within the concavity of the internal calyx (IntCX). GLB, globulus; DVNBL, unequally dividing neuroblast; NBL, neuroblast; DVGMC, dividing ganglion mother cell.

Figure 23. Photomicrograph of a frontal section through the brain of a 50-day-old embryo of P. americana, showing the inner formation centre of the globuli cells. Note a dividing neuroblast in the centre. Lettering as in Figure 22.

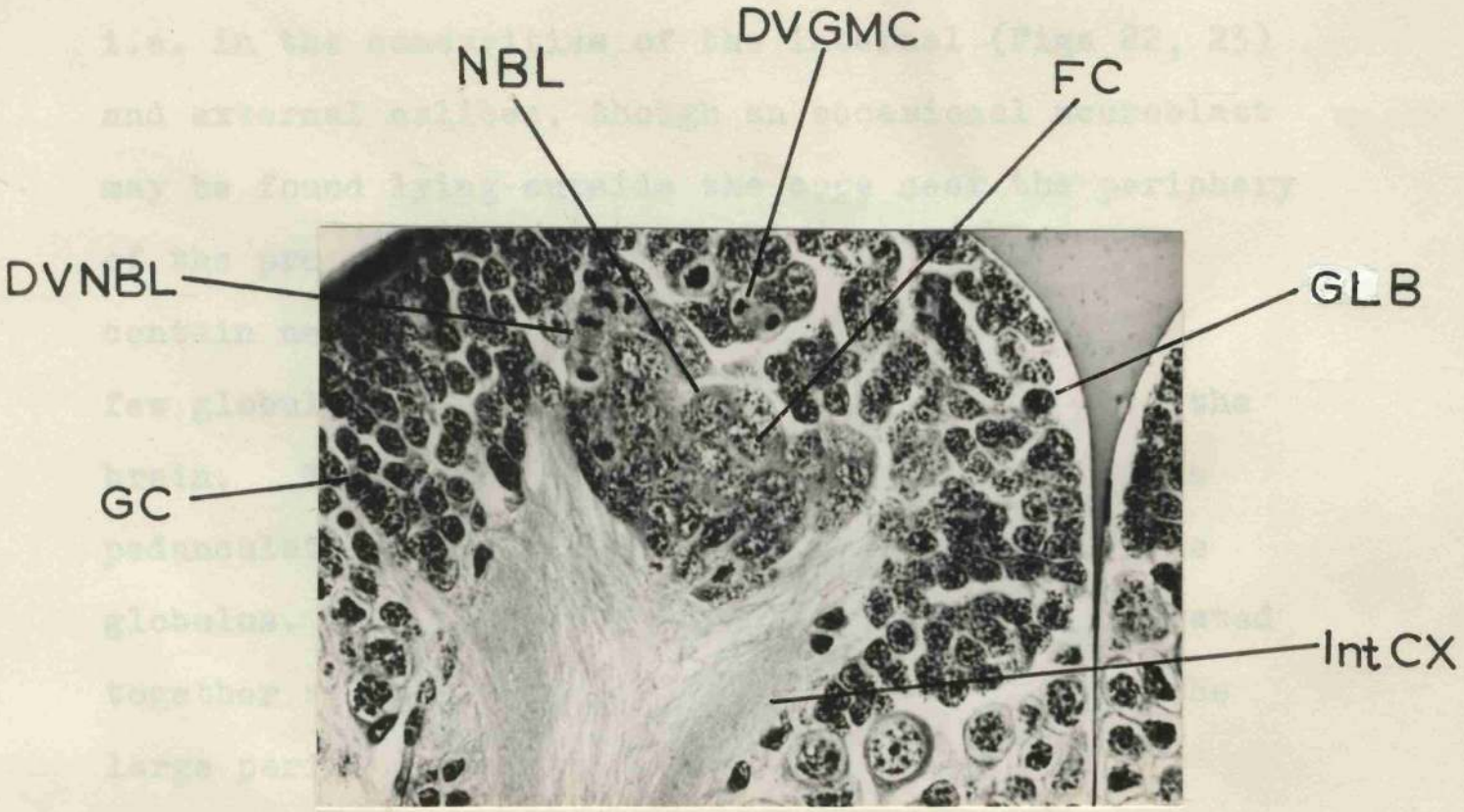


Figure 22.

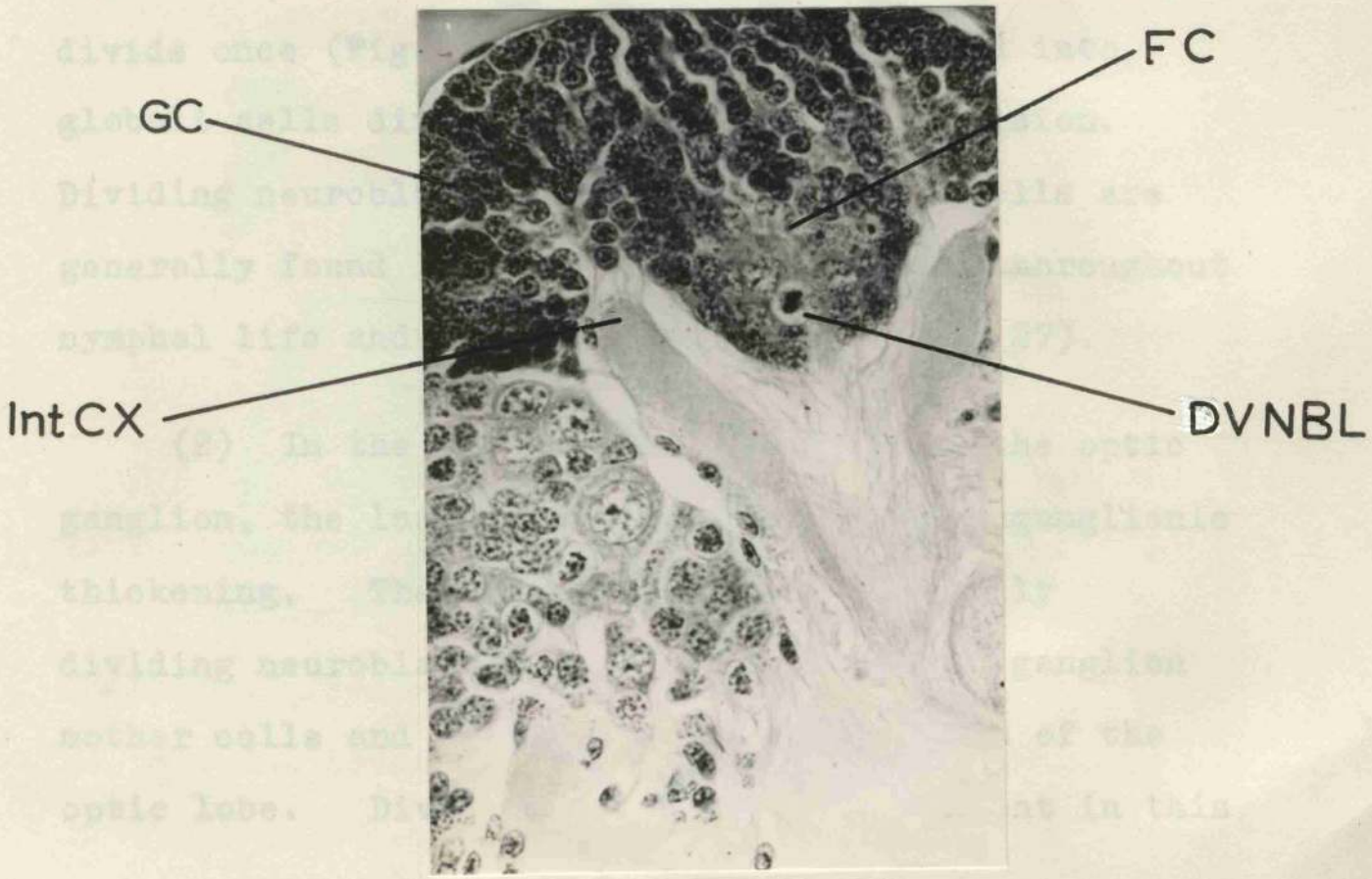


Figure 23.

i.e. in the concavities of the internal (Figs 22, 23) and external calices, though an occasional neuroblast may be found lying outside the cups near the periphery of the protocerebrum. These formation centres contain neuroblasts, ganglion mother cells and a few globuli cells (or neurons) of this region of the brain. The region of the brain containing corpus pedunculatum and its globuli cells is known as the globulus. The neuroblasts found here are aggregated together and are certainly smaller in size than the large peripheral ones but the former also divide unequally (Fig. 24) like the latter (Fig. 25). The smaller product or ganglion mother cell undergoes equal division to form two daughter cells which may divide once (Fig. 26) or may be transformed into globuli cells directly without further division. Dividing neuroblasts and ganglion mother cells are generally found in these formation centres throughout nymphal life and even in some adults (Fig. 27).

(2) In the two formation centres of the optic ganglion, the lamina ganglionaris and intraganglionic thickening. These contain smaller unequally dividing neuroblasts, resting neuroblasts, ganglion mother cells and clusters of ganglion cells of the optic lobe. Dividing cells are also present in this

Figure 24. Photomicrograph of a frontal section through the brain of a second instar nymph of P. americana killed 9½ days after the moult. Note an unequally dividing neuroblast (DVNBL) in the concavity of one cup and an equally dividing ganglion mother cell (DVGMC) in the other cup.

Figure 25. Photomicrógraph of a frontal section through the brain of an 11-day-old embryo of P. americana, showing an unequally dividing neuroblast (DVNBL) in the optic lobe.

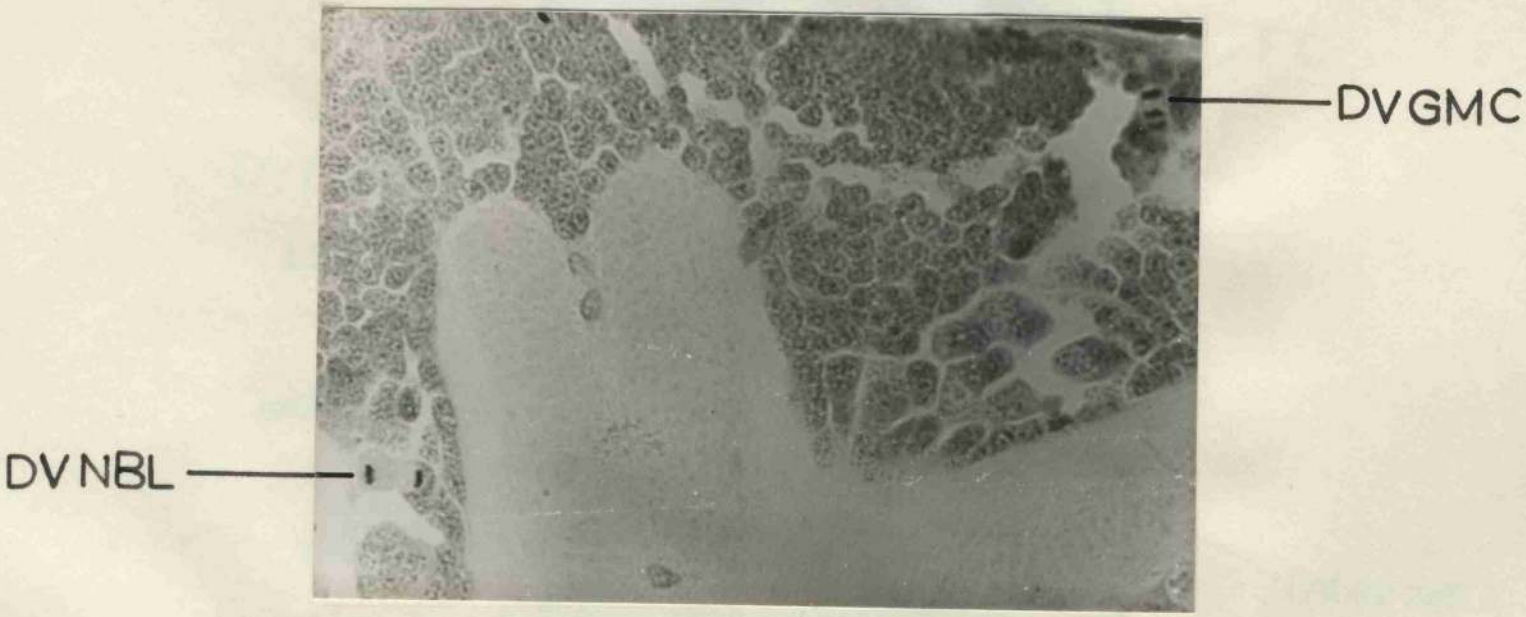


Figure 24.

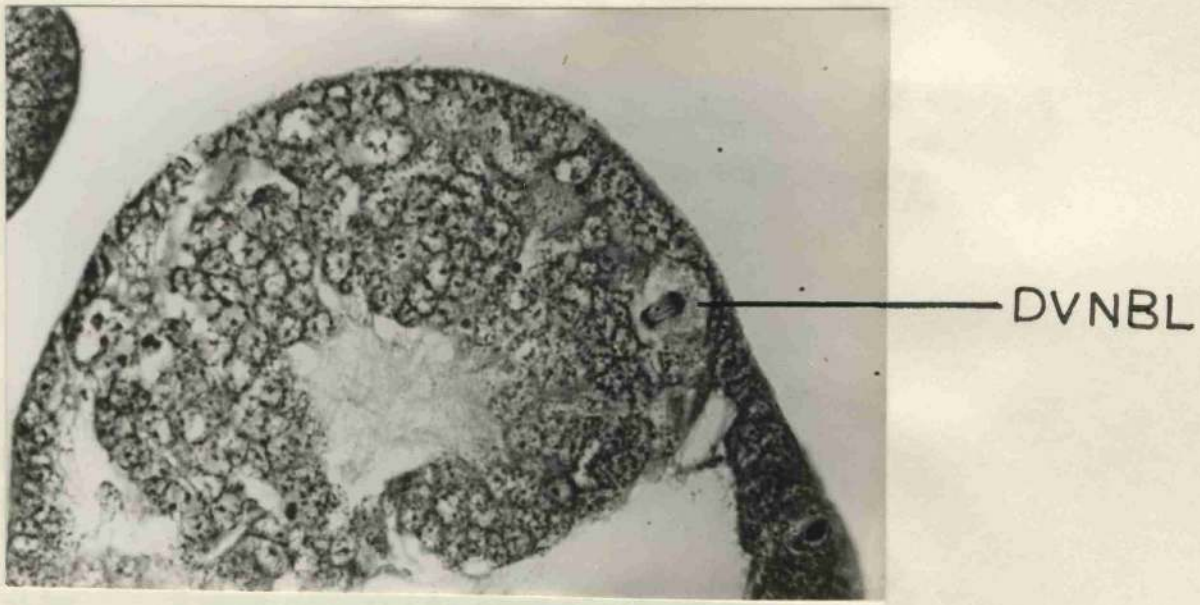


Figure 25.

Figure 26. Photomicrograph of a frontal section through the brain of a 10½-day-old first instar nymph of P. americana, showing the internal calyx (cup) of the mushroom body and the inner formation centre (FC) of the globuli cells within this cup. See dividing neuroblast (DVNBL), dividing ganglion mother cell (DVGMC) and still smaller cells dividing, which are probably dividing daughter cells (DVDC).

Figure 27. Photomicrograph of a frontal section through the brain of an adult P. americana, killed 126 days after the last moult, showing the inner formation centre (FC) of globuli cells lying in the concavity of the internal calyx (Int.CX). Note a dividing neuroblast (DVNBL). Figure 27 is at a higher magnification than Figure 26.

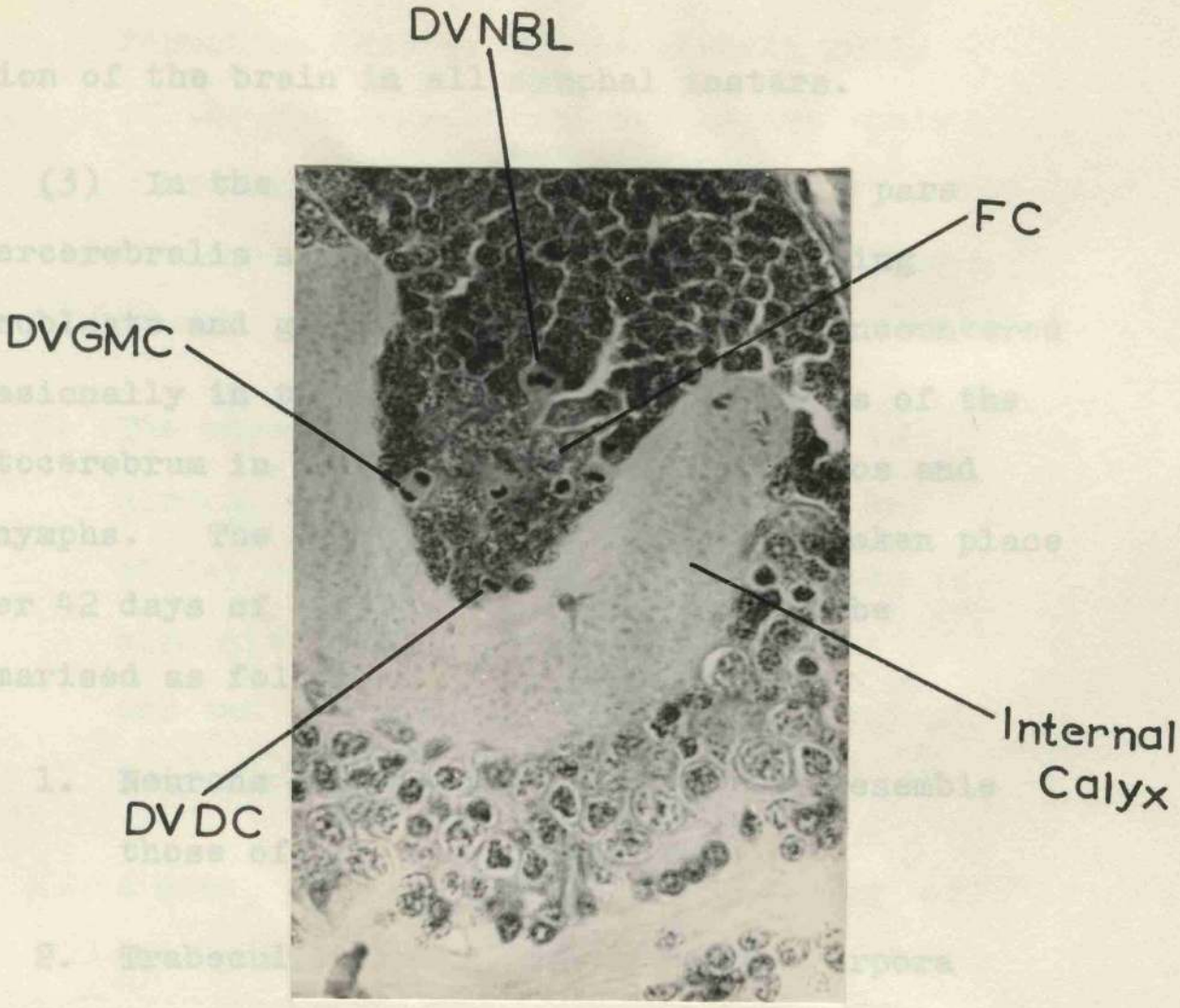


Figure 26.

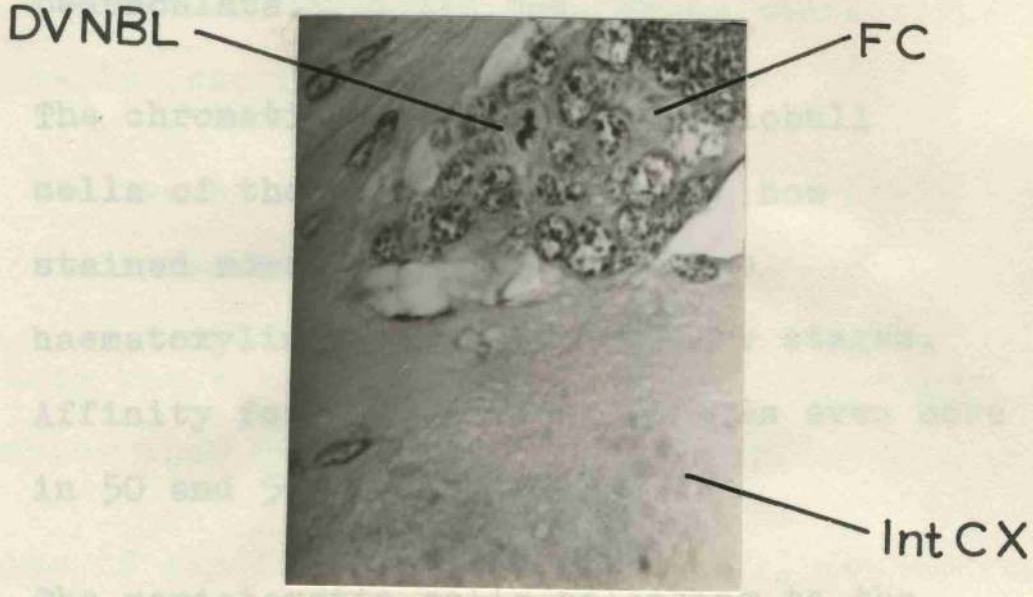


Figure 27.

region of the brain in all nymphal instars.

(3) In the meristematic region of the pars intercerebralis and pars lateralis. Dividing neuroblasts and ganglion mother cells are encountered occasionally in the median and lateral parts of the protocerebrum in the 42-day and later embryos and in nymphs. The major changes which have taken place after 42 days of embryonic development can be summarised as follows:

1. Neurons are now well defined and resemble those of a first instar nymph.

2. Trabeculae and cauliculi of the corpora pedunculata have increased greatly in size, as have the cups of the corpora pedunculata.

3. The chromatin material of the globuli cells of the mushroom bodies is now stained more intensely by Goat's haematoxylin than in the earlier stages. Affinity for this stain increases even more in 50 and 54-day embryos.

4. The meristematic cells belonging to the

formation centres of the globuli cells of the mushroom bodies now assume their customary position in the concavity of the internal and external cups as described above.

5. The upper part of the central body is already divided into 8 segments in the 30-day embryo, and now the lower part is also divided into 8 compartments and the two ventral tubercles of the central body have appeared.

6. A group of small glia cells staining darkly with haematoxylin appears ventral to the neuropile of the proto-:cerebrum and lies in between the ventral neurosecretory cells and nervus connectivus cells (see Chapters 1 and 4).

7. The neuropiles of the deuto-, and trito-:cerebrum have become glomerular.

In all these respects the brain of the 42-day embryo now resembles that of a first instar nymph.

Chains of ganglion cells (Figs 20, 21) projecting inwards from the periphery can be clearly seen, because

of the presence of interspaces between them, up to the tenth day of embryonic development but, as the number of ganglion cells increases and the brain in consequence increases in size, actual chains of cells become more difficult to distinguish. At first a ganglion cell is so poor in cytoplasm that it appears to consist only of a nucleus with a single nucleolus and sparse chromatin, but presently the volume of cytoplasm in the perikaryon increases as does the chromatin content of the nucleus. At the same time, the axon, which starts as a fine protoplasmic extension, is developing. The first sign of such axon development is detectable in the brain of a 10-day embryo but neurons similar to those in first instar nymphal brains cannot be found until the thirtieth day. These neurons continue to differentiate, cytoplasmic volume, chromatin content of nucleus and thickness of axon increasing until the forty-second day. Beyond this point there is little further change in neurons during embryonic life.

Section II: Brain

As in other orthopteran (old classification) insects the brain of P. americana is formed by three pairs of ectodermal thickenings at the anterior end of the embryo, consisting of neuroblasts and dermatoblasts, which give rise to the proto-, deuto-, and

tritocerebrum. The formation of these ectodermal thickenings is followed by the development and differentiation of the neuroblasts. Each half of the protocerebrum of an embryo of P. americana, like that of Xiphidium (Wheeler, 1893), Locusta (Roonwal, 1937), and Melanoplus (Baden, 1937) is further divisible into three parts, viz., the first protocerebral lobe (or optic lobe or optic ganglion), the second protocerebral lobe, and the third protocerebral lobe, counting from the outside (Figs 20, 21). These parts of the brain, which can be distinguished for the first time in 10-day embryos, will be considered separately.

(1) Protocerebral lobes

a) First protocerebral lobe or optic lobe

In the 10-day embryo the first protocerebral lobe has already separated from the overlying eye plate which consists of dermatogenic cells from which the future retinal cells of the compound eye will develop. Unequally dividing neuroblasts and resting neuroblasts can both be found at the periphery of the optic lobe, in the region of the lamina ganglionaris and at the junction of the optic lobe and the second protocerebral lobe in the region of origin of the intraganglionic thickening. Smaller equally dividing and resting ganglion mother cells lie below the neuroblasts in the

Figure 28. Photomicrograph of a frontal section of the optic lobe of a 10-day-old embryo of P. americana, showing the optic lobe or 1st protocerebral lobe (IPRC), 2nd protocerebral lobe (IIPRC), intraganglionic thickening (INT) between the 1st and the 2nd proto-:cerebral lobes. Note also an unequally dividing neuroblast (DVNBL) in the optic lobe, and the eye plate (EP).

Figure 29. Photomicrograph of a frontal section through the head of an 11-day-old embryo of P. americana, showing 1st, 2nd and 3rd protocerebral lobes (I, II, IIIPRC), the intraganglionic thickening (INT), eye plate (EP), antennal coelom (AntCoel), neuroblast (NBL), the protocerebral bridge (PBr) and the central body (CB).

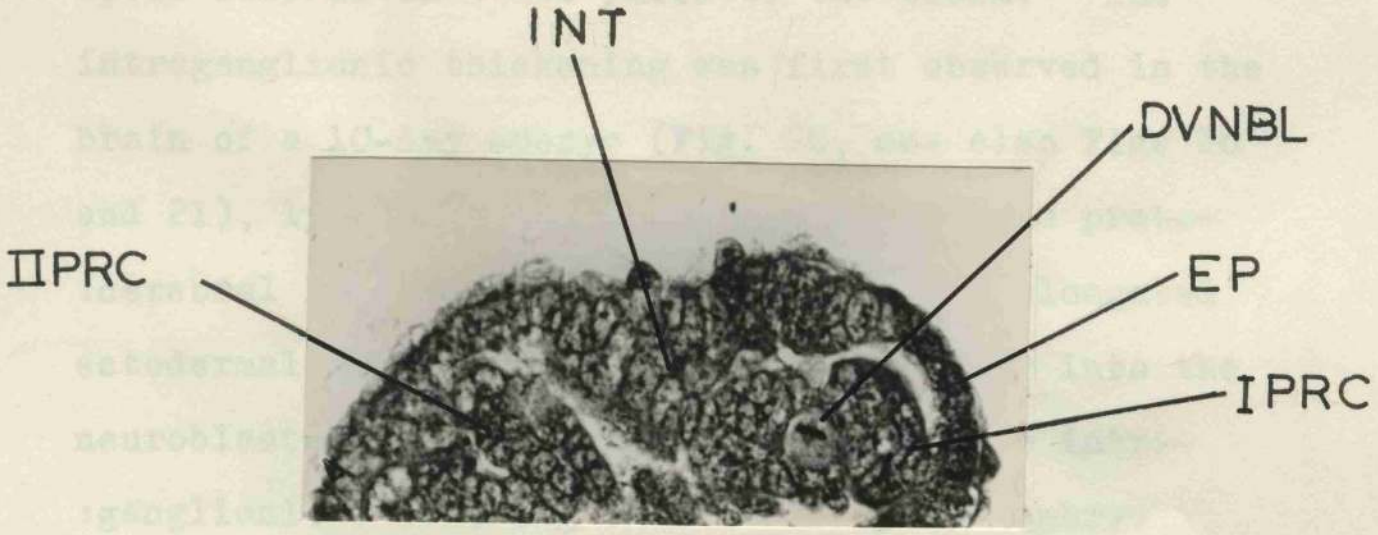


Figure 28.

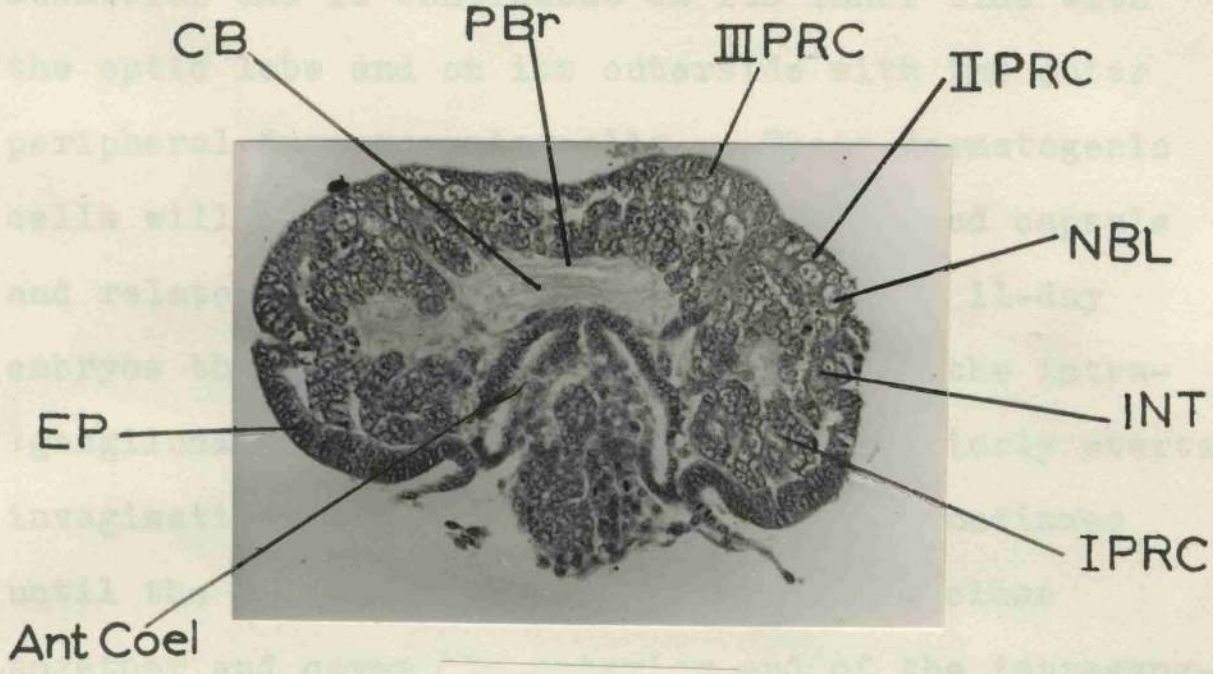


Figure 29.

optic lobe as in other parts of the brain. The intraganglionic thickening was first observed in the brain of a 10-day embryo (Fig. 28, see also Figs 20 and 21), lying between the first and second proto-:cerebral lobes. It consists mainly of elongated ectodermal cells which differentiate later into the neuroblasts of this region. However, the intra-:ganglionic thickening of the 10-day-old embryo already contains a few differentiated neuroblasts some of which are dividing unequally in characteristic manner. These extend to the base of this body. In 10, 11 and 15-day embryos, this structure extends downwards and is continuous on its inner side with the optic lobe and on its outerside with the outer peripheral dermatogenic cells. These dermatogenic cells will eventually give rise to the head capsule and related integumental structures. In 11-day embryos that part of the head with which the intra-:ganglionic thickening is connected anteriorly starts invaginating (Fig. 29) and this process continues until the sides of the invagination come close together and cover the anterior end of the intragang-:libnic thickening. The intraganglionic thickening and the dermatogenic cells which lie over it are still in contact. Later, however, when the head capsule

Figure 30. Photomicrograph of a frontal section of the brain of a 20-day-old embryo of P. americana, showing the optic lobe (OPT), the intraganglionic thickening (INT), a dividing neuroblast (DVNBL) in the intraganglionic thickening, large peripheral neuroblasts (NBL) of 2nd protocerebral lobe, an unequally dividing neuroblast (DVNBL) in the region of lamina ganglionaris (LG), a large neuroblast in the optic lobe, the eye plate (EP), the neuropile of lamina ganglionaris (NpLG), medulla externa (ME), medulla interna (MI) (the medulla interna is not obvious in this section), the neural lamella (NL), and the perineurium cell (PC).

Figure 31. Photomicrograph of a frontal section through the optic lobe of a 35-day-old embryo of P. americana, showing the intraganglionic thickening which is still near the periphery of the brain. See a dividing neuroblast in this body. Other structures as in Figure 30. Lettering as in Figure 30.

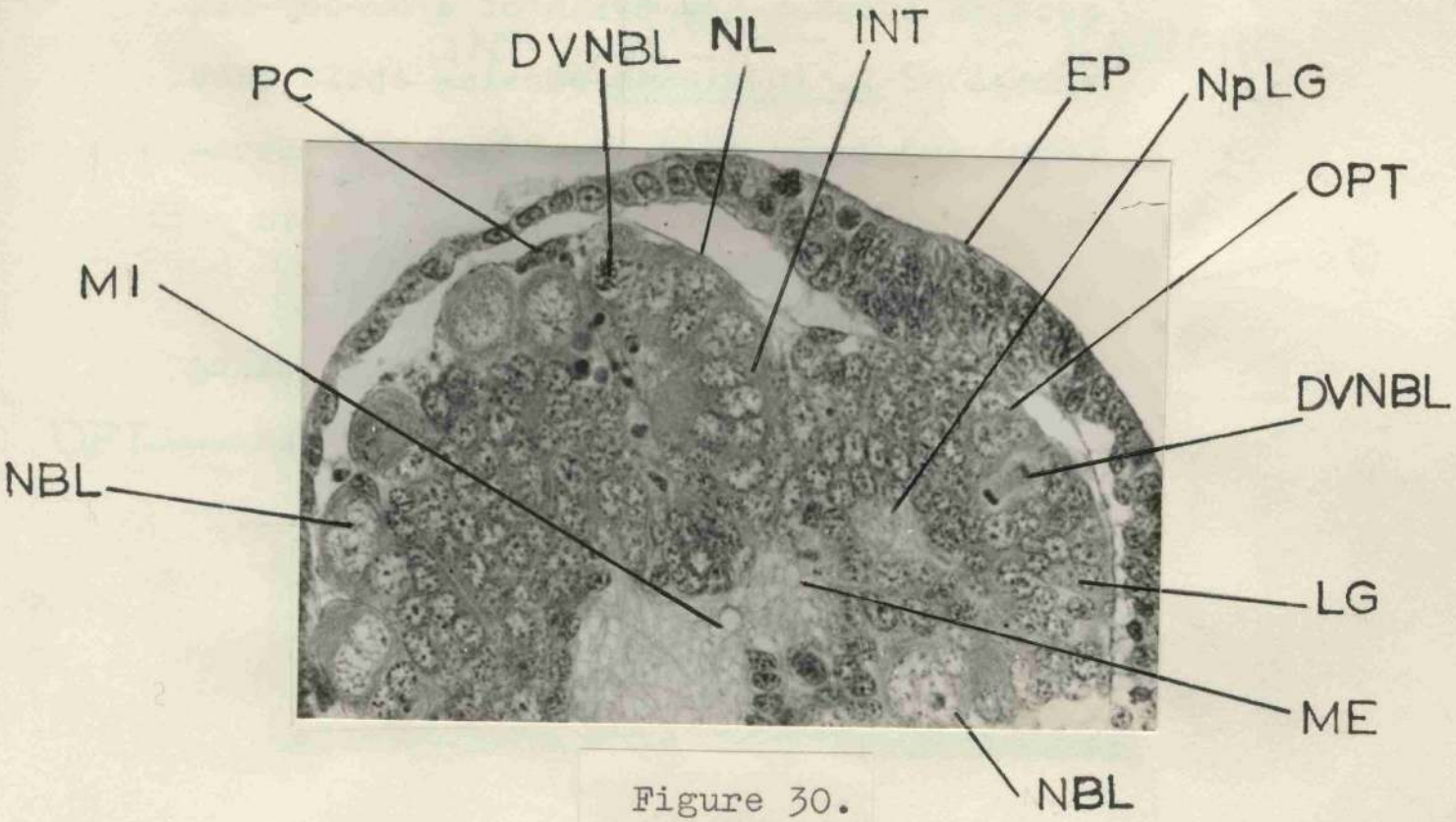


Figure 30.

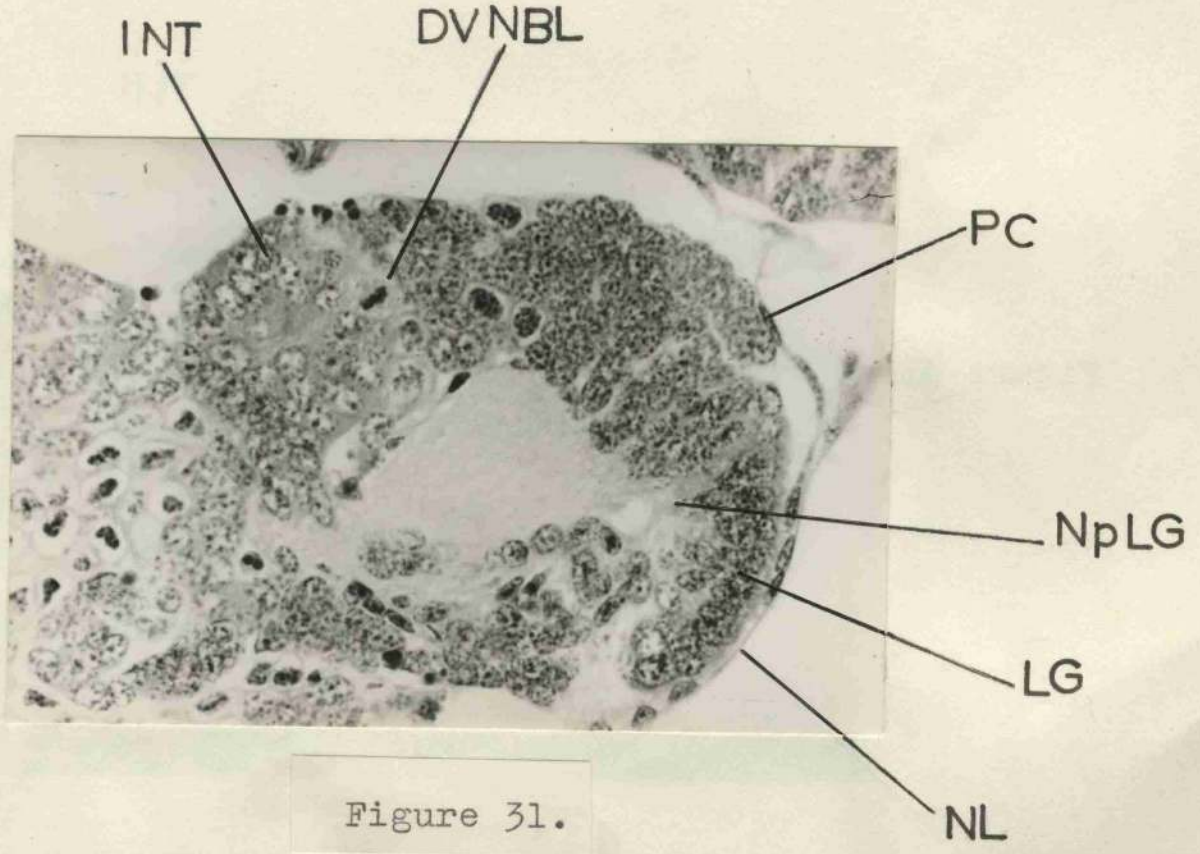


Figure 31.

Figure 32. Photomicrograph of a posterior frontal section through the brain of a 42-day-old embryo of P. americana, showing optic lobe (OPT) and fused IInd and IIIrd protocere-:bral lobe. INT, the intraganglionic thickening lying between the medulla externa and medulla interna and extending as a narrow band of meristematic cells up to the third protocerebral lobe. DVNBL, dividing neuroblast; Ext.C, external calyx; GC, globuli cells; G, glia cells.

Figure 33. Photomicrograph of a frontal section through the brain of a 42-day embryo, showing the intraganglionic thickening (INT). Same magnification as Figures 30 and 31.

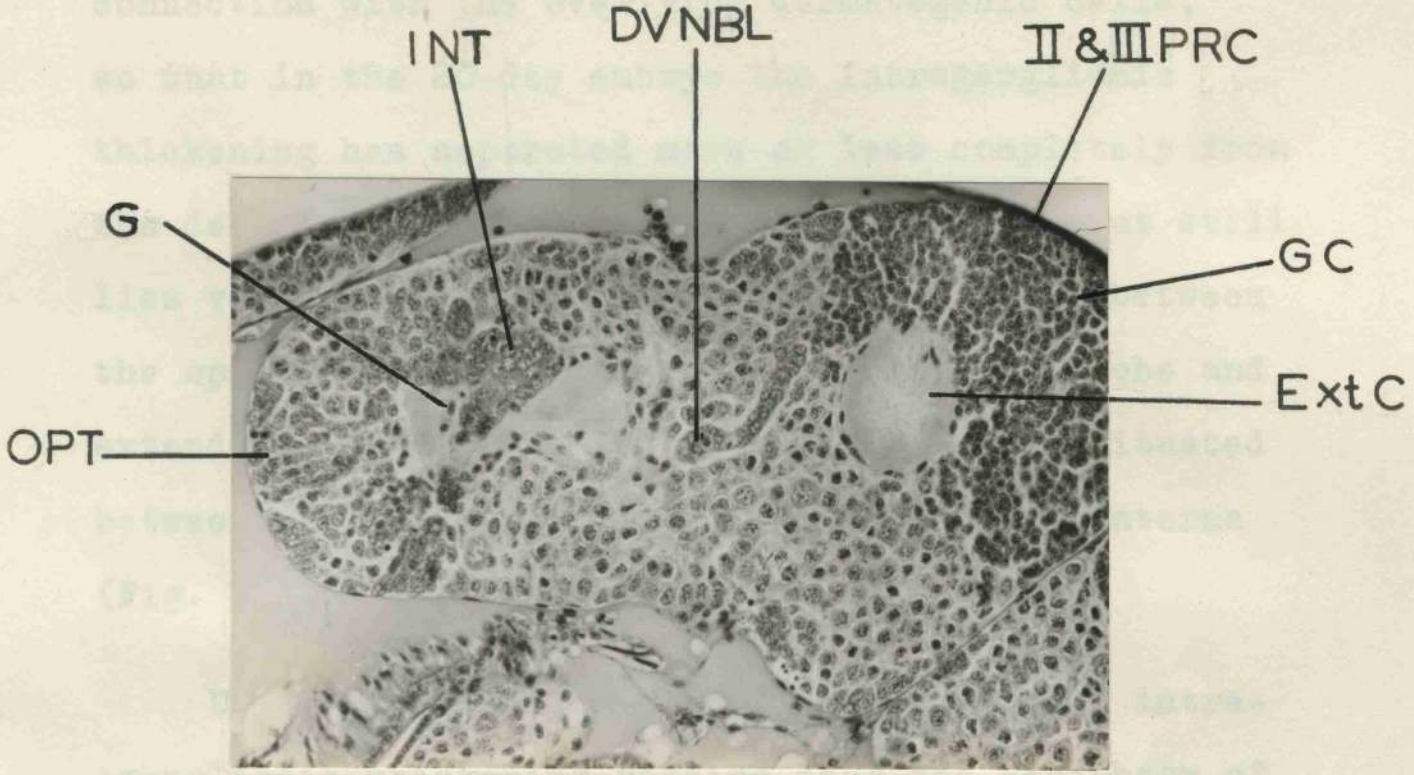


Figure 32.

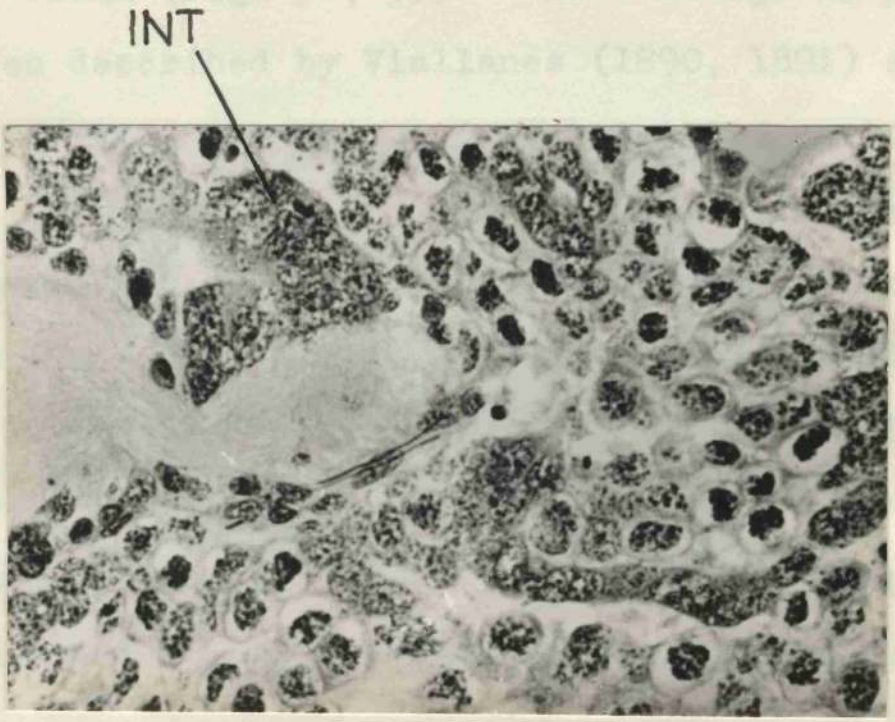


Figure 33.

is formed, the intraganglionic thickening loses its connection with the overlying dermatogenic cells, so that in the 20-day embryo the intraganglionic thickening has separated more or less completely from the dermatogenic cells of the head capsule, but still lies very near to the periphery of the brain between the optic lobe and the second protocerebral lobe and extends downwards so that its lower part is situated between the medulla externa and the medulla interna (Fig. 30).

Up to the thirty-fifth day (Fig. 31) the intra-:ganglionic thickening remains near the periphery of the brain, but by the forty-second day it has become confined to the region between the medulla externa and the medulla interna and thus appears to lie deep in the brain (Figs 32, 33). This change in position has been described by Viallanes (1890, 1891) and Wheeler (1893) as a migration, but it is really due to the differentiation of all the anterior peripheral meristematic cells of the intraganglionic thickening into the ganglion cells of the optic lobe. The inner region lodged between the external and the internal medullary masses of the optic ganglion remains as a zone of meristematic cells throughout the later embryonic and post embryonic development,

Figure 34. Photomicrograph of a frontal section through the region of the lamina ganglionaris of the optic lobe of a 20-day-old embryo of P. americana showing an unequally dividing neuro-:blast (DVNBL), a dividing ganglion mother cell (DVGMC), and a still samller dividing cell which is probably a dividing daughter cell (DVDC).

Figure 35. Photomicrograph of a frontal section through the optic lobe of a newly moulted nymph of B. orientalis, showing an unequally dividing neuroblast (DVNBL) in the region of the lamina ganglionaris.

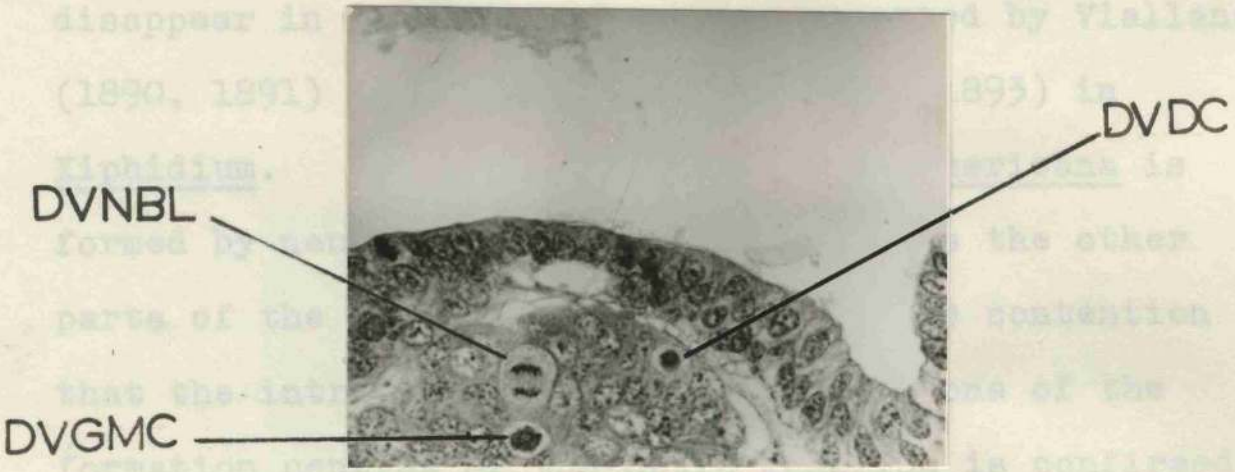


Figure 34.

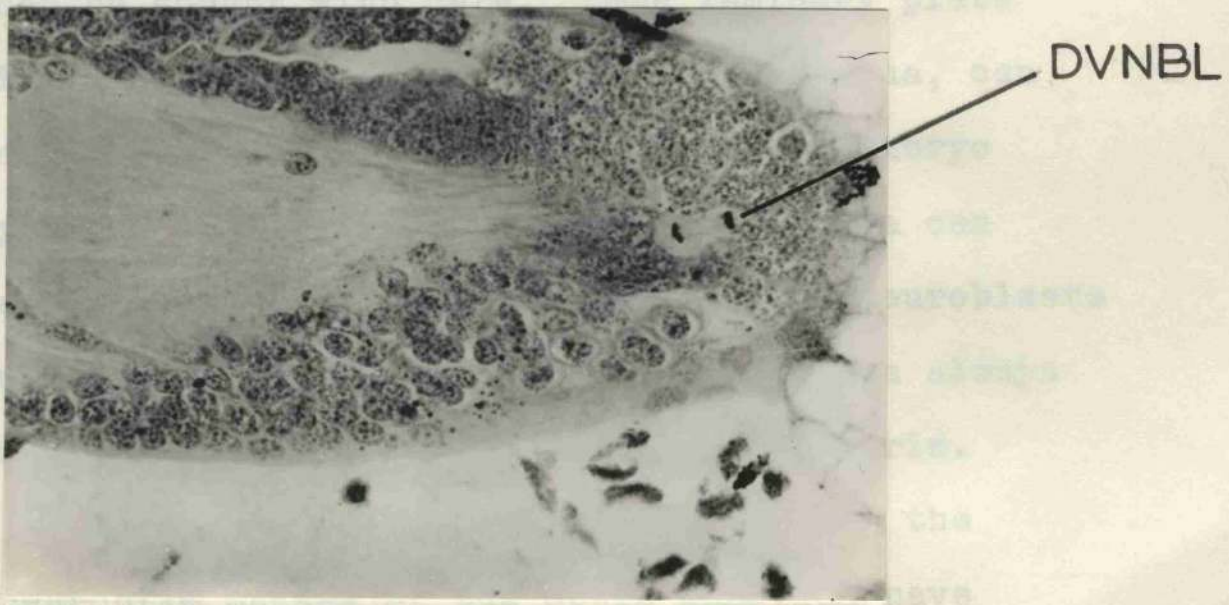


Figure 35.

as the meristematic cells of the lamina ganglionaris.

Thus the intraganglionic thickening does not disappear in the late embryo, as reported by Viallanes (1890, 1891) in Mantis and by Wheeler (1893) in Xiphidium. The optic ganglion of P. americana is formed by neuroblasts in the same way as the other parts of the brain and so Bauer's (1904) contention that the intraganglionic thickening is one of the formation centres of the optic ganglion is confirmed.

The post-retinular fibres, first seen in the 10-day embryo, are very thin thread-like protoplasmic structures connecting the cells of the eye plate with the lamina ganglionaris of the optic lobe. They become slightly thicker in later embryos, and appear only in one or two serial sections of a head so that they must be sought with care. The laminary plate or lamina ganglionaris, and the medulla externa, can be first observed in the brain of an 11-day embryo and, by the twentieth day, the medulla interna can also be distinguished. Unequally dividing neuroblasts and equally dividing ganglion mother cells can always be found associated with the lamina ganglionaris. (Figs 34, 35.) In 42, 50 and 54-day embryos the three neuropile masses of the optic ganglion have

Figure 36. Photomicrograph of a frontal section of the optic lobe of a 54-day-old embryo of P. americana, showing the half moon shaped neuropile mass of the lamina ganglionaris (NpLG), the bigger three-layered mass of the medulla externa (ME), the small medulla interna (MI), external chiasma (EC) and the internal chiasma (IC). OPT, optic lobe; DT, deutocerebrum.

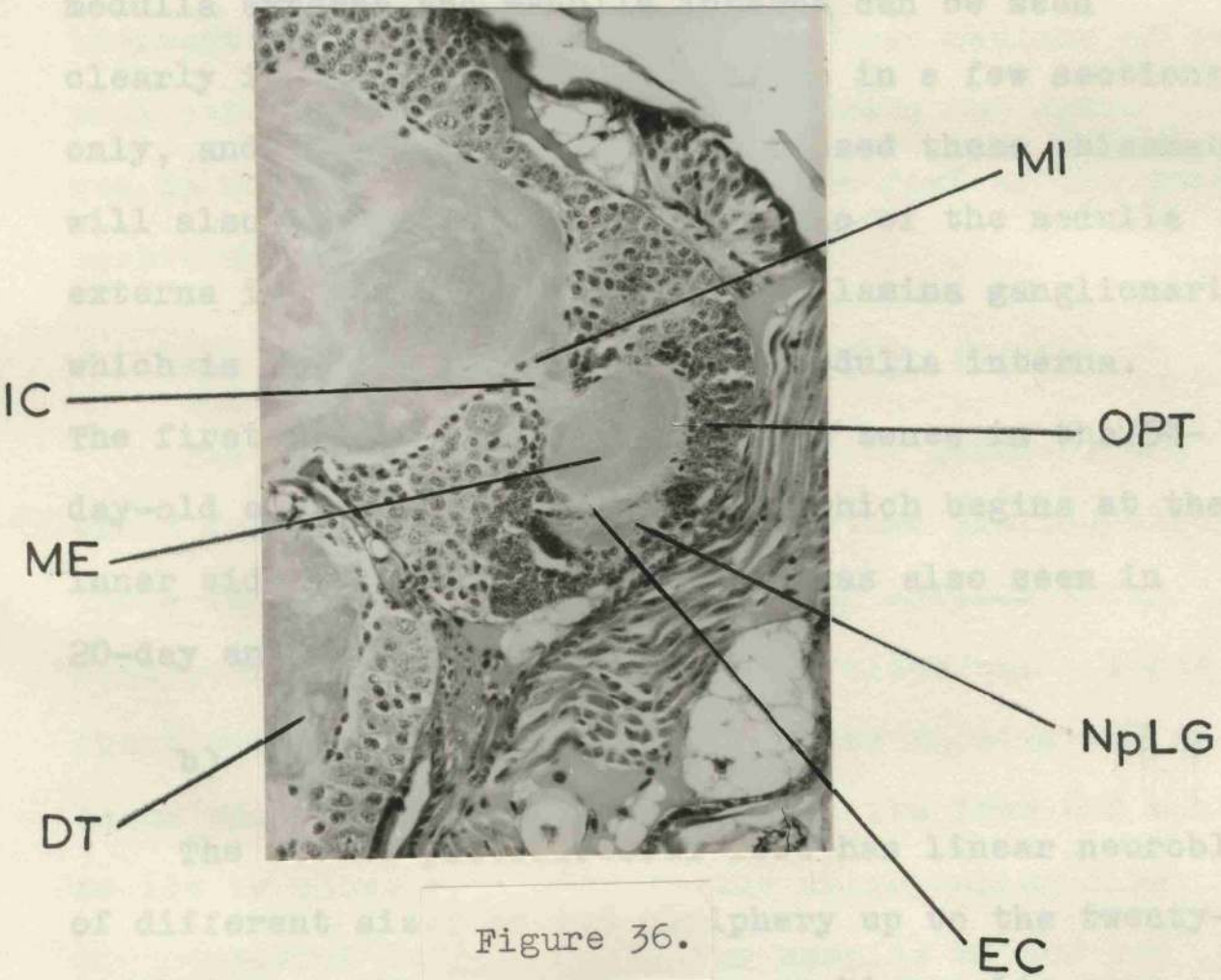


Figure 36.

increased in size (Fig. 36). The first crossing (or external chiasma) of nerve fibres lying between and connecting the neuropile of the lamina ganglionaris and the medulla externa, and the second chiasma (or internal chiasma) lying between and connecting the medulla externa and medulla interna can be seen clearly in 20-day and older embryos in a few sections only, and if these sections are missed these chiasmata will also be missed. The neuropile of the medulla externa is larger than that of the lamina ganglionaris which is in turn larger than the medulla interna. The first can be divided into three zones in the 54-day-old embryo. The optic nerve which begins at the inner side of the medulla interna was also seen in 20-day and older embryos.

b) Second protocerebral lobe

The second protocerebral lobe has linear neuroblasts of different sizes at its periphery up to the twenty-fifth day. These peripheral neuroblasts lie very close to the intraganglionic thickening; so close, indeed, that it seems as if they are penetrating it (Fig. 30). The internal medullary mass and the optic nerve seem to arise from the second protocerebral lobe of P. americana in a way similar to that described by

Roonwal (1937) in Locusta.

The second protocerebral lobe gradually separates from the dermatogenic cells of the head and starts merging with the third protocerebral lobe. This merger is complete in 30-day and older embryos and it is thereafter difficult to demarcate these regions of the protocerebrum from one another, whereas the optic lobes can be easily distinguished from the rest of the proto-:cerebrum.

c) Third protocerebral lobe (Figs 37, 38)

The median or third protocerebral lobes of the brain form the major part of the future protocerebrum. There are neuroblasts at their outer surface which disappear during late embryonic development. About the eleventh day these lobes increase in size and each bends upon itself (Fig. 38) so that its free end comes to lie in close proximity to the neighbouring first protocerebral lobe. The outer margins of the two third lobes are adjacent to one another at the bends but separated by a median line which divides the brain into two equal halves. This central region of the protocerebrum is the median pars intercerebralis and in 11-day and later embryos it has neuroblasts arranged vertically along its periphery near the median line.

Figure 37. Photomicrograph of a frontal section through the head of a 10-day-old embryo, showing 1st, 2nd and 3rd protocerebral lobes (I, II, III PRC), stomodaeum (ST), antennary coelom (ANT COEL).

Figure 38. Photomicrograph of a frontal section through the head of an 11-day-old embryo, showing the brain. Note that each 3rd protocerebral lobe has bent upon itself. NBL, neuroblast; DVNBL, dividing neuroblast; NpPRC, neuropile of the protocerebrum. (See the protocerebral bridge in Figure 29.)

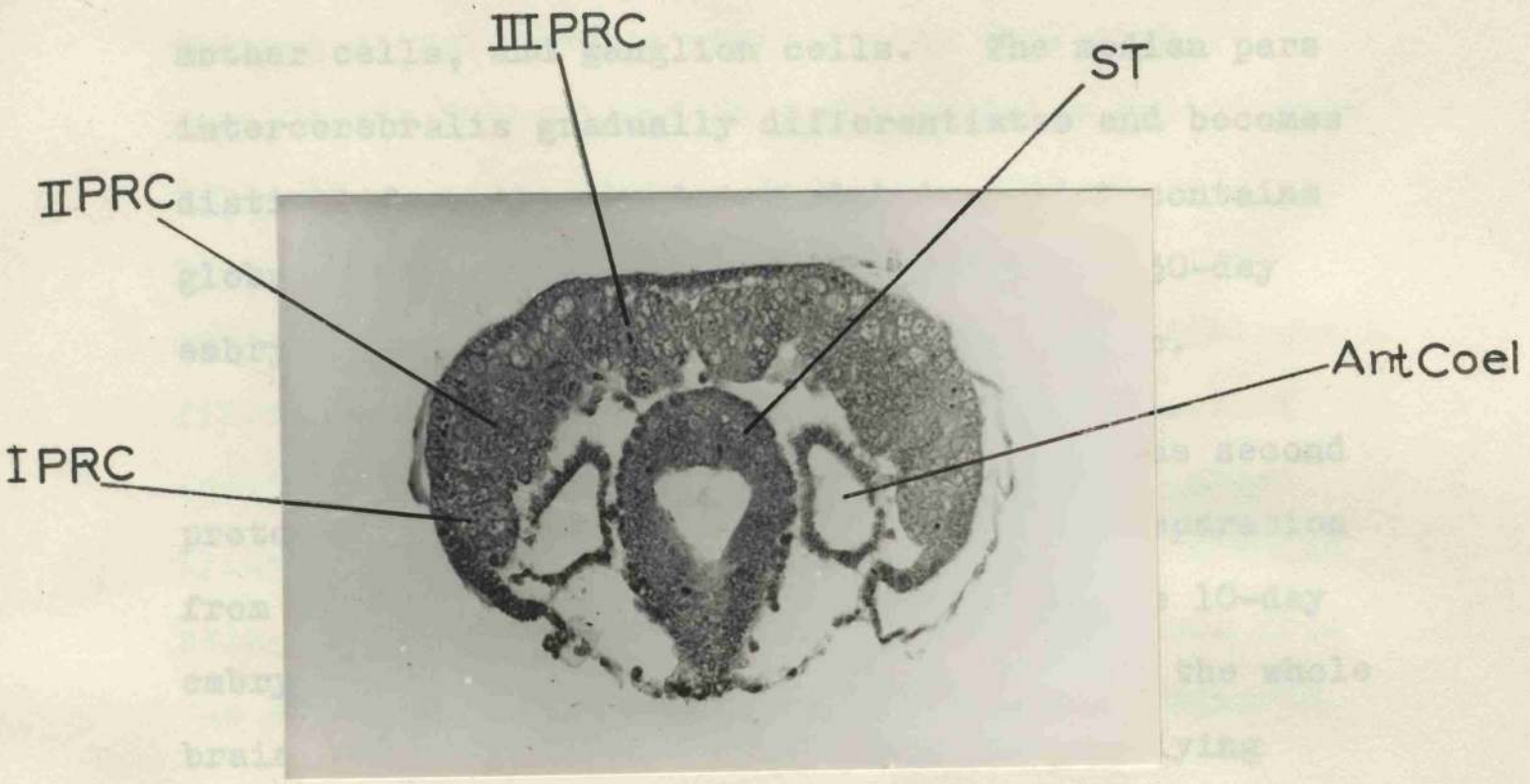


Figure 37.

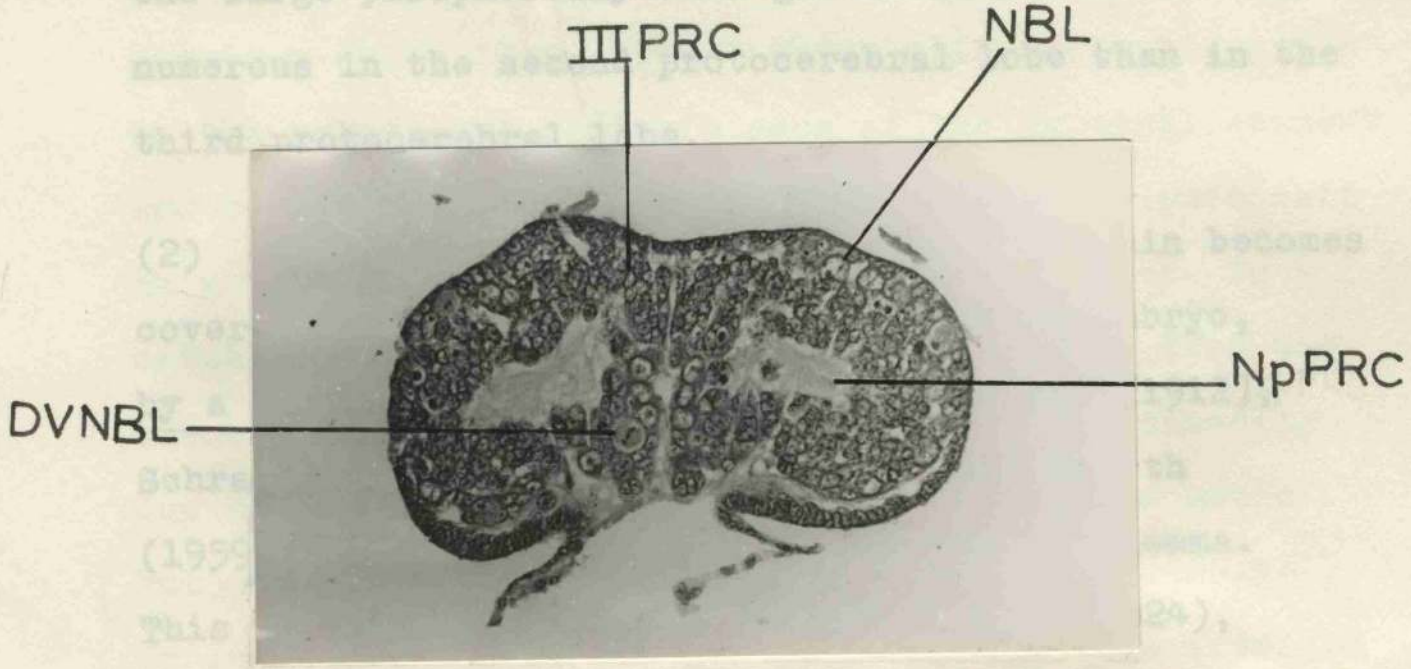


Figure 38.

In addition to the neuroblasts, there are ganglion mother cells, and ganglion cells. The median pars intercerebralis gradually differentiates and becomes distinct from the overlying globulus which contains globuli cells. This change begins in the 30-day embryo and is completed in the 42-day embryo.

The third protocerebral lobe, unlike the second protocerebral lobe, shows slight signs of separation from the overlying dermatogenic cells in the 10-day embryo. However, in 20 and 25-day embryos the whole brain has virtually separated from the overlying dermatogenic cells, though in places they may be connected by means of protoplasmic strands to the neural lamella of the brain. In 20 and 25-day embryos, the large peripherally-arranged neuroblasts are more numerous in the second protocerebral lobe than in the third protocerebral lobe.

(2) <u>The perilemma and glia cells</u>. The brain becomes covered, for the first time in the 20-day embryo, by a sheath which, in agreement with Feyer (1912), Schrader (1938), Scharrer (1939), Wigglesworth (1959a), and others, will be termed the perilemma. This is the layer Heymons (1895), Nelson (1924), Roonwal (1937), Baden (1937), and others, called the

outer neurilemma. It consists of a very thin outer fibrous neural lamella which stains pink with paraldehyde fuchsin and an inner cellular perineurium (Fig. 30). According to Baccetti (1955, 1956), Hess (1958) and Smith & Wigglesworth (1958), the neural lamella is laminated and consists of collagen fibrils. Hoyle (1952) and Hess (1958) state that 'perilemma' should be used for the cellular layer lying below the neural lamella, the word perineurium being discarded, but this unnecessary departure from the accepted meaning of the terms should be ignored.

In the brain of the 10-day embryo a small number of glia cells can be seen for the first time, at the lower surface of and within the rudimentary protocerebral neuropile which lies below the dorsal ganglion cells (Fig. 39). These glia cells lie close to the evanescent coelomic sacs of the antennal segment and resemble the mesodermal cells of the coelomic sacs in shape, size and staining properties. There is a notable increase in the number of glia cells from the twentieth day on, an increase which is not primarily due to mitotic activity of these cells in the brain but is due to the fact that the brain of the embryo is invaded by these mesodermal elements, this invasion

Figure 39. Photomicrograph of a frontal section through the head of a 10-day-old embryo, showing the 1st, 2nd and 3rd protocerebral lobes (I, II, III PRC), the rudimentary protocerebral neuropile (NpPRC) and few glia cells (G) associated with it. ANT COEL, evanescent antennary coelom.

Figure 40. Photomicrograph of a frontal section through the head of a 20-day-old embryo of P. americana, showing the invasion of brain by mesodermal cells which form the perineurium and glia cells. Note also the thread-like protoplasmic processes of the dermatogenic cells. ST, stomodaeum; G, glia cells; P, perineurium cell.

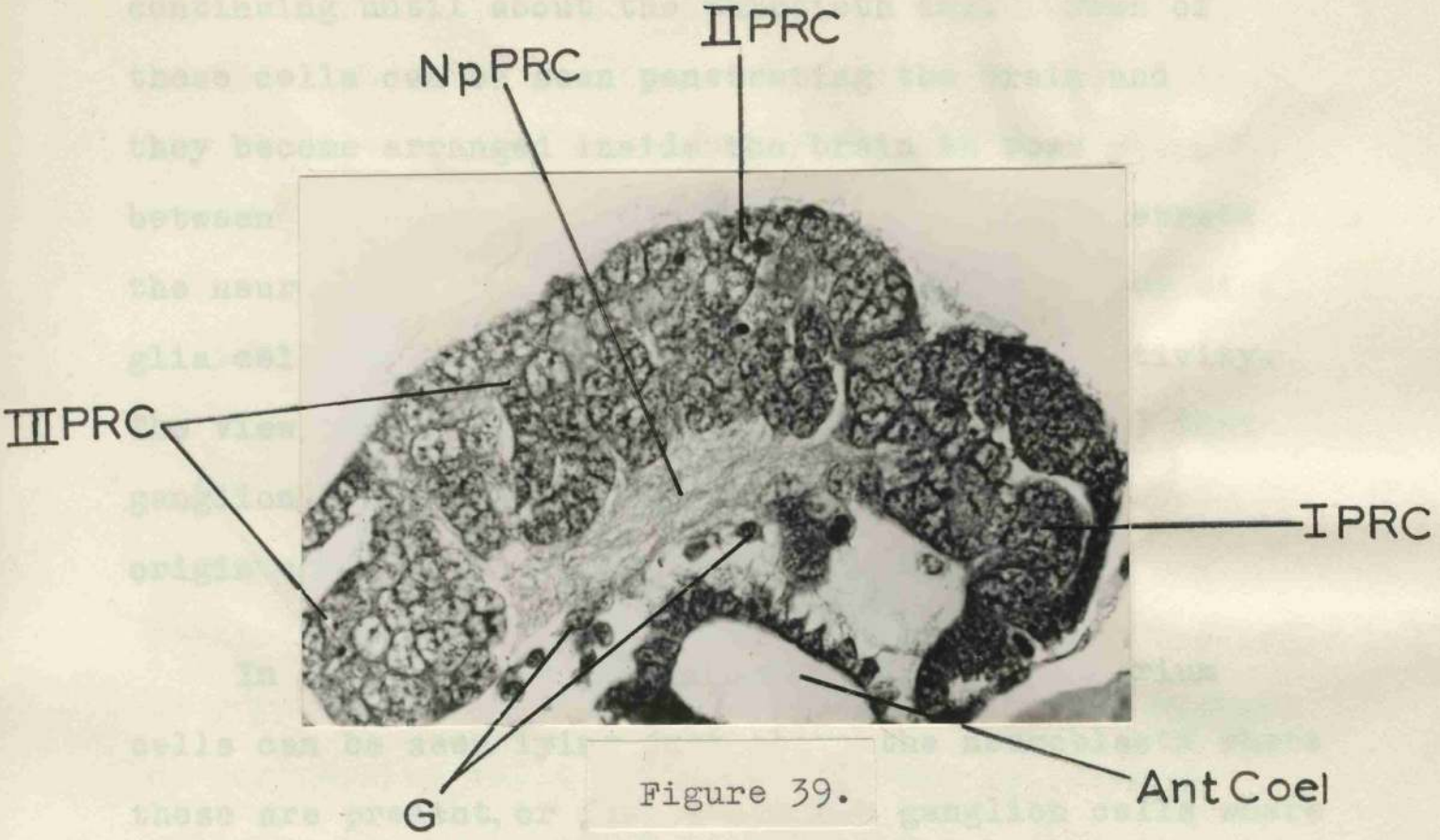


Figure 39.

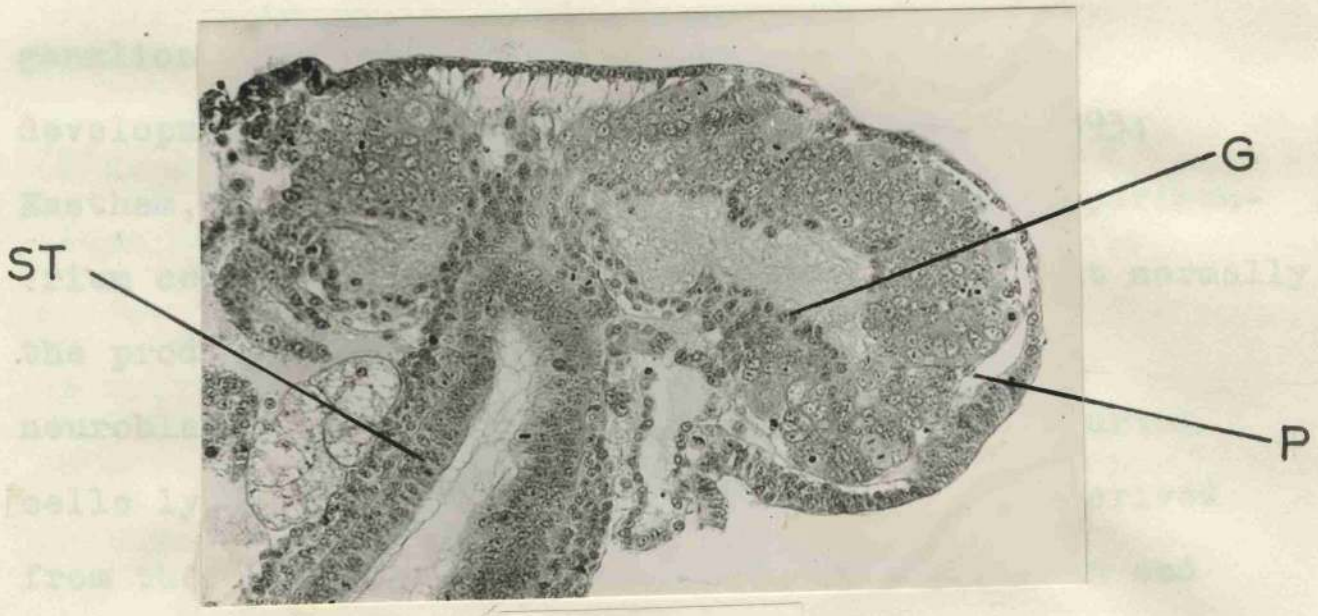


Figure 40.

continuing until about the thirtieth day. Rows of these cells can be seen penetrating the brain and they become arranged inside the brain in rows between the ganglion cells and surround and penetrate the neuropile (Fig. 40). Increase in the number of glia cells at later stages is due to mitotic activity. The view of Pflugfelder (personal communication) that ganglion cells and glia cells have a common origin cannot be accepted.

In the brain of 20-day embryos the perineurium cells can be seen lying just above the neuroblasts where these are present, or just above the ganglion cells where these occur at the periphery of the brain. The close association of perineurium cells with the peripheral ganglion cells at this and subsequent stages of development has led some observers (Wheeler, 1893; Eastham, 1930; Roonwal, 1937) to suppose that perineu-:rium cells are derived from ganglion cells, but normally the products of the division of the peripheral neuroblasts lie internal to them so that perineurium cells lying above the neuroblasts can only be derived from them if they have migrated to that position and have undergone a rapid and drastic differentiation such as a great reduction in size, and changes in

staining properties and shape. On the other hand, perineurium cells so closely resemble glia cells in shape, size and staining properties, that it seems probable that they have a common origin and differentiate later for different functions. The first appearance of perineurium cells in the embryonic brain coincides in time with the invasion of the brain by mesodermal cells some of which, as has already been suggested, become glia cells. It is now postulated that the remainder arrange themselves on the surface of the brain as perineurium cells. Subsequent increase in numbers of perineurium cells is due to mitotic activity by cells in the perineurium itself. Baden (1937) also ascribed a mesodermal origin to perineurium cells, tracing them to the cephalic coelomic sacs in Melanoplus. At the convex parts of the brain the perineurium cells (Fig. 30) are small, more or less flat, elongate, and not close to one another, but in other places, in the concave regions for example, these cells are oval, round or cylindrical and arranged in several layers. Such an arrangement has also been reported by Scharrer (1939) in the nymphs and adults of P. americana.

Though numerous dark-stained small glia cells lie all along the periphery of the neuropile of 20-day

and older embryos, no definite continuous membrane, i.e. internal neural lamella, as reported by Wheeler (1893), Heymons (1895) and others, could be seen. Baden (1937) also could not find such an internal neural lamella surrounding the neuropile in Melanoplus, and discarded the possibility of its existence on the grounds that if such an internal membrane existed it would have to possess numerous pores for the passage of each and every axon.

The perineurium cells are thought to secrete the neural lamella, though according to Wigglesworth (1956) "contribution by haemocytes remains a possibility". In P. americana embryos a few haemocytes with pink granules were observed lying in the space outside the brain, between the neural lamella and the head capsule, but haemocytes were not observed closely applied to the outer surface of the neural lamella. In 20 and 25-day embryos the neural lamella of the brain is connected to the dermatogenic cells of the head by means of the protoplasmic processes of the latter which extend ventrally to reach the outer surface of the brain. These processess anastomose and are closely applied to the outer surface of the neural lamella while the perineurium cells are closely applied to its inner surface. The dermatogenic cells lose

their protoplasmic connections with the neural lamella of the brain about the thirtieth day when a definite head capsule is formed enclosing the brain.

(3) The Punktsubstanz

About the tenth day of development the ganglion cells start to produce thin protoplasmic processes (axons) which become interwoven with each other and form a thin fibrous network linking the first, second and third protocerebral lobes of each half of the brain with each other. By the eleventh day the neuropile connecting both the third protocerebral lobes has been formed. This median neuropile band (or mass) is known as the protocerebral bridge. This neuropile of the protocerebrum, is referred to by German authors as Punktsubstanz and so it can be stated that Punktsubstanz appears as early as the tenth day of development (see Figs 29, 38 and 39).

The protocerebral neuropile of a 10-day embryo lies ventral to the dorsal ganglion cells and is so thin that it has been seen in only one or two of the whole series of sections through the head. The brain of a 10-day-old embryo of P. americana resembles the stage F embryonic brain of Xiphidium (Wheeler, 1893) in that the protocerebral lobes are at the same stage of

development but at this stage the brain of Xiphidium, according to Wheeler, lacks Punktsubstanz. Yet in the first stage at which he noted the presence of a neuropile this was as fully developed as the neuropile of an 11-day embryo of P. americana in which the number of ganglion cells is now greater, the brain is now more compact, and the neuropile mass of the protocerebrum is thicker and embedded in the mass of ganglion cells. So it seems probable that a thin neuropile in stage F embryos of Xiphidium escaped Wheeler's notice.

The neuropile of the proto-, deuto- and tritocerebrum increases gradually during embryonic and post-embryonic development. The neuropile structure of the brain gradually become differentiated during embryonic development and apparently the only change which they undergo during post-embryonic development is increase in size.

(4) The corpora pedunculata (mushroom bodies)

There are two corpora pedunculata in the protocerebrum. These are fibrous or neuropile structures formed by the axons of the ganglion or globuli cells of the globulus region of the protocerebrum. Each of the corpora consists of a trabecula, a cauliculus, a main peduncle, and two calices (or cups) filled with globuli cells.

Figure 41. Photomicrograph of a frontal section through the brain of a 25-day embryo of P. americana, showing rudiment of calyx (Cx) and peduncle (PED). PRC, protocerebrum; DTC, deutocere-:brum; TRC, tritocerebrum; NBL, neuroblast; DVNBL, dividing neuroblast; P, perineurium cell; NL, neural lamella; G, glia cell; GANGC, ganglion cell; H, head capsule; Np, neuropile.

Figure 42. Photomicrograph of a sagittal section through the brain of a 30-day embryo of P. americana, showing rudimentary internal calyx (Int.CX) and external calyx (Ext.CX) and globuli cells (GLC).

Figure 43. Photomicrograph of a frontal section through the brain of a 42-day embryo, showing the calices of the corpora pedunculata. Lettering as in Figures 41 and 42.

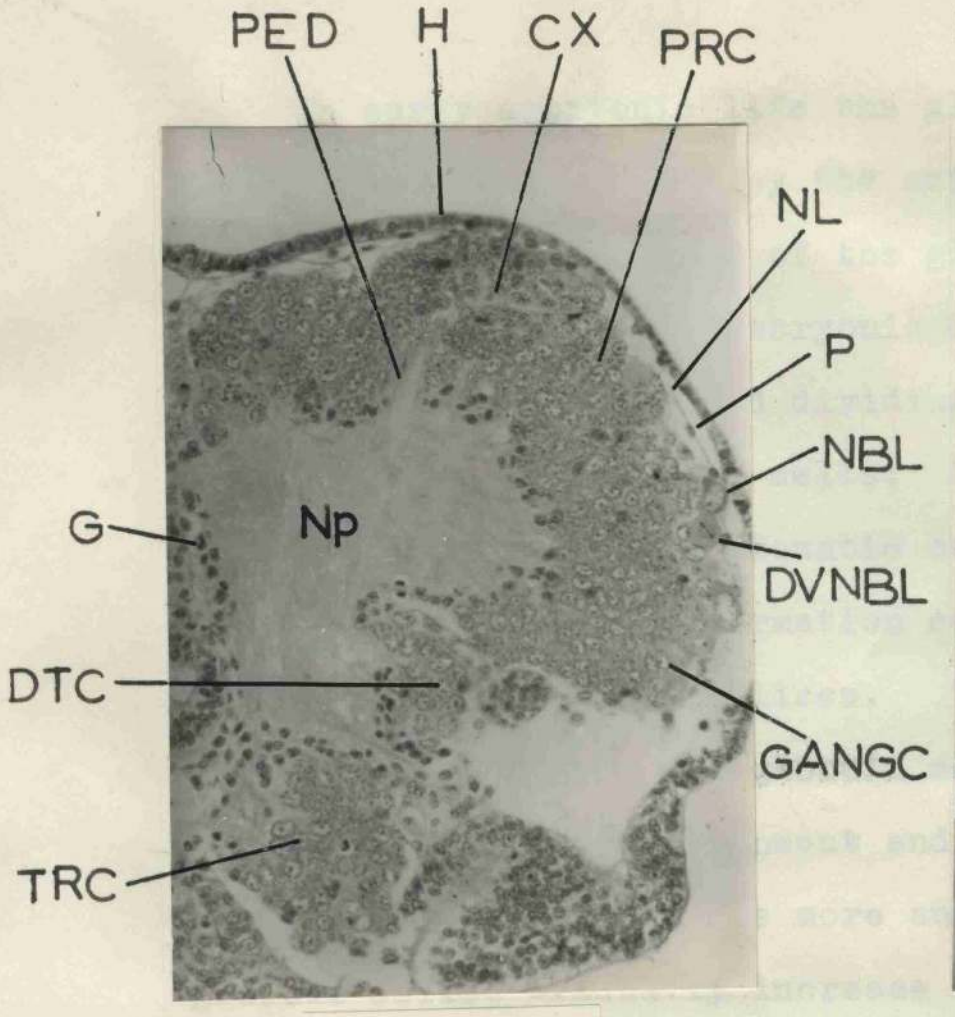


Figure 41.

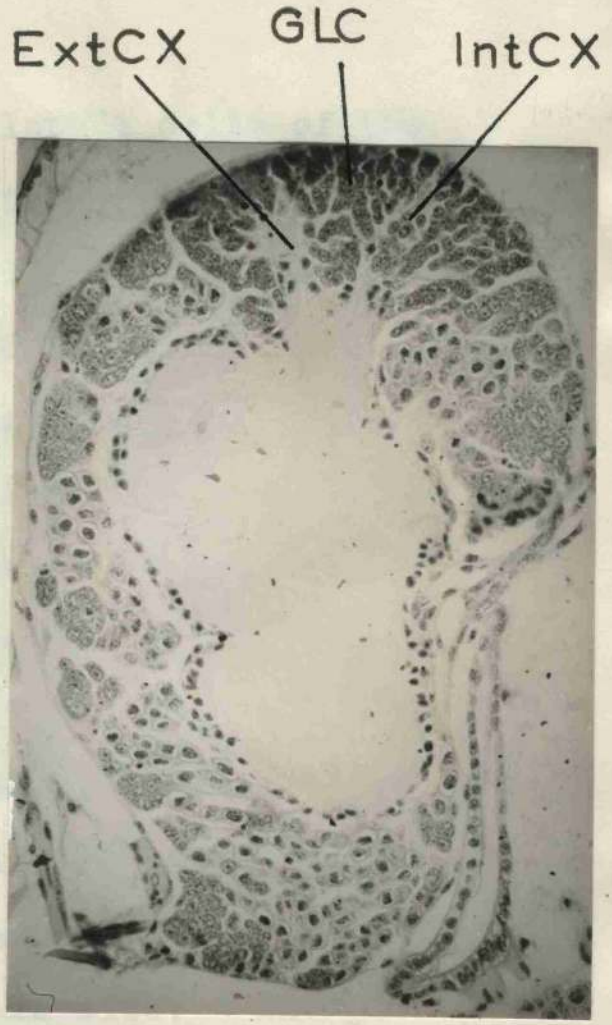


Figure 42.

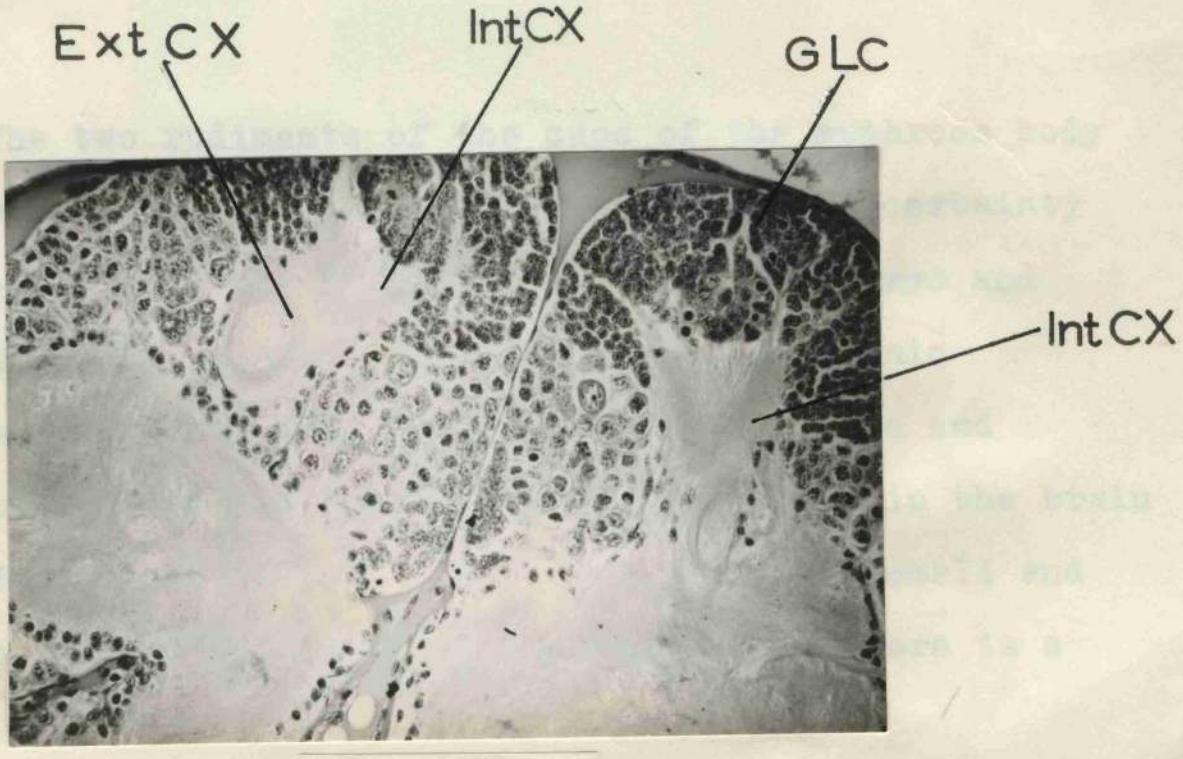


Figure 43.

In early embryonic life the globuli cells of the mushroom body are formed by the activity of peripheral protocerebral neuroblasts of the globulus region and up to the thirtieth day of embryonic development the globulus is filled with resting and dividing neuroblasts, ganglion mother cells, and globuli cells; but in 42-day and older embryos most of the meristematic cells of the globulus become confined to the formation centres in the concavities of the now well formed calices. These centres continue to produce more and more globuli cells throughout embryonic and post-embryonic development and so the corpora peducnulata, which receive more and more axons from the globuli cells, gradually increase in size. The globuli cells are round in shape, smaller than most of the other ganglion cells of the brain but bigger than the glia cells.

The two rudiments of the cups of the mushroom body with rudimentary stalks were first seen with certainty in each half of the brain of a 25-day-old embryo and they increase in size during the later embryonic development (Figs 41, 42, 43). The trabecula and cauliculus become visible for the first time in the brain of a 30-day-old embryo. They are then very small and remain small till the thirty-fifth day but there is a

Figure 44. Photomicrograph of a frontal section through the brain of a 35-day embryo, showing the trabecula (TR), central body (CB), the upper part of which is divided into 8 segments but the lower part is still undivided; protocerebral bridge (PBr), the ventral commissure of the ventral mass (VC) and glia cells (G).

Figure 45. Photomicrograph of a frontal section through the brain of a 42-day embryo, showing trabecula (TR), cauliculus (CAU), central body (CB) whose upper, as well as lower, part is divided into 8 compartments. Note also the glomeruli (GL) in the neuropile of the antennary lobe. Figure 45 is at a lower magnification than Figure 44.

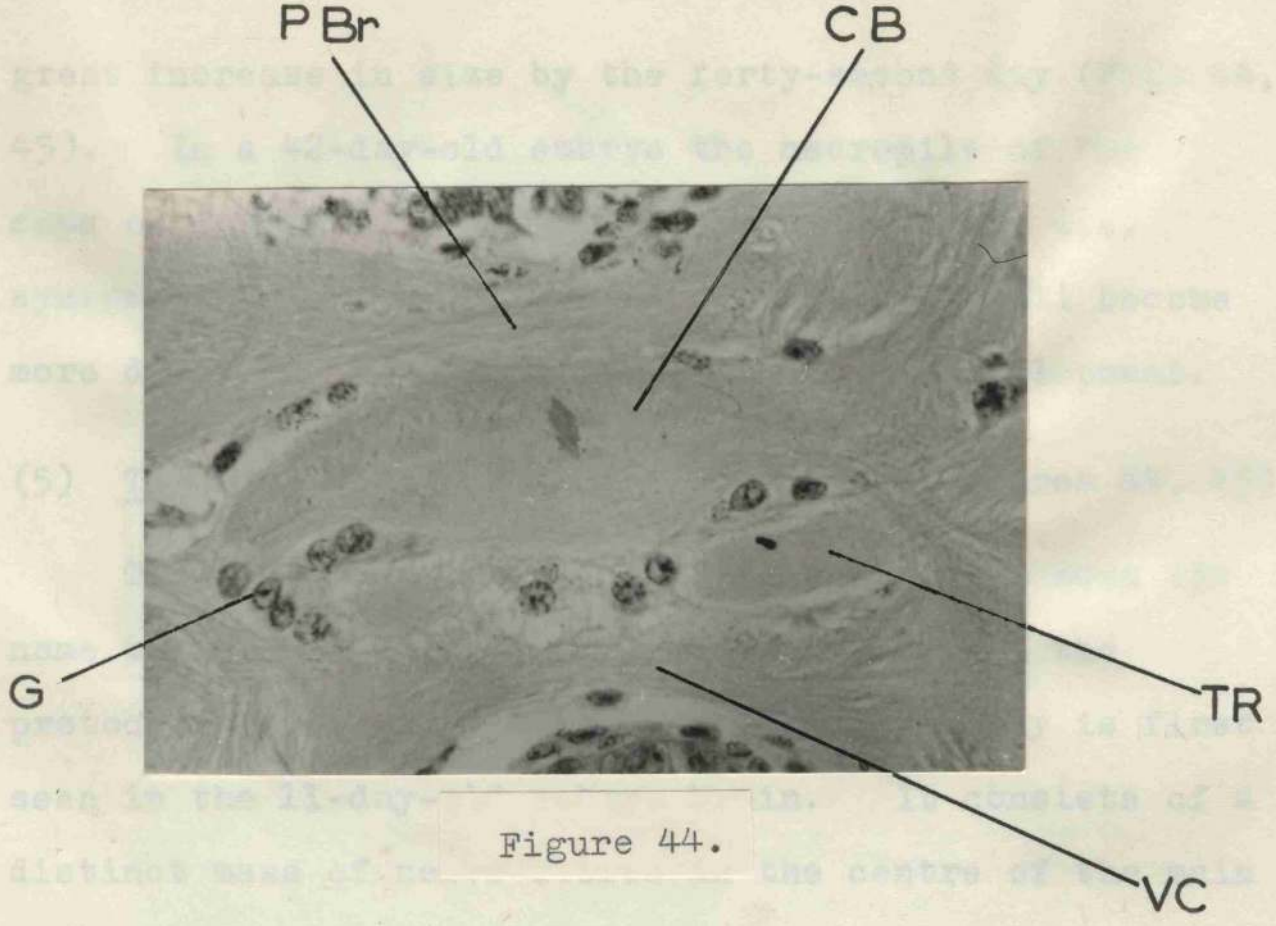


Figure 44.

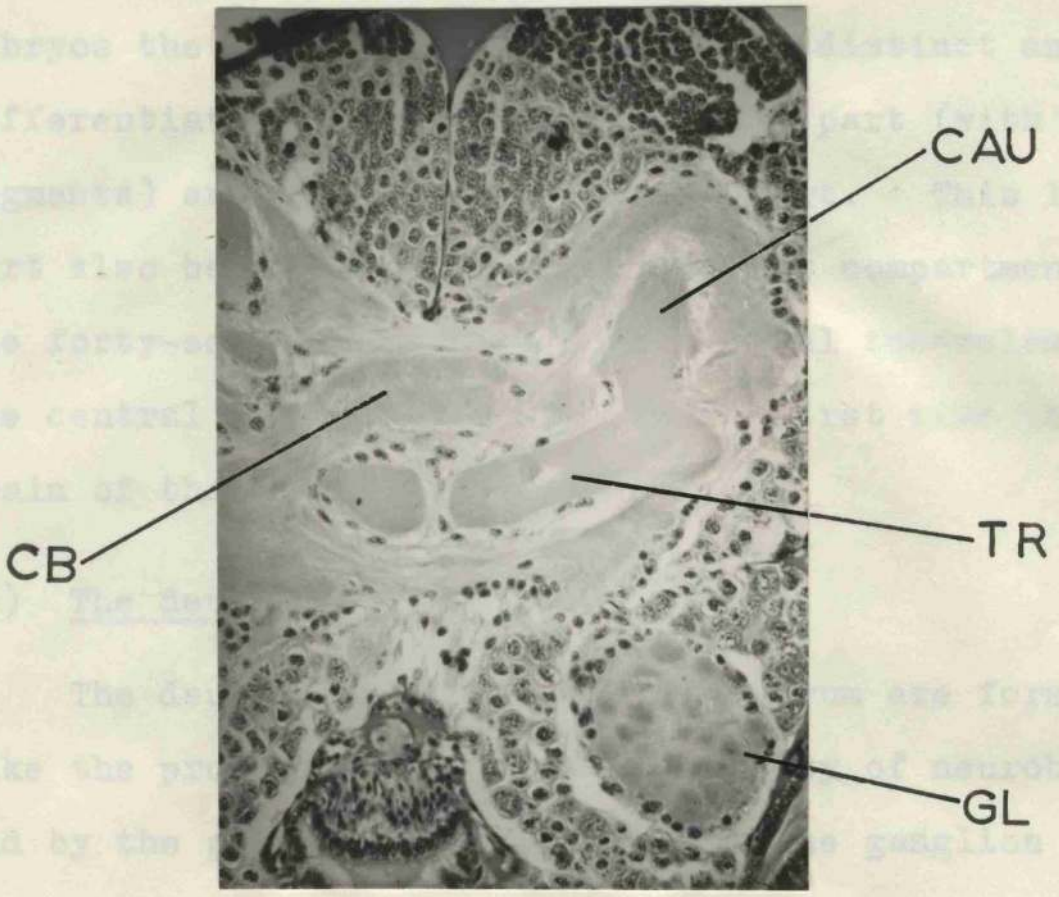


Figure 45.

great increase in size by the forty-second day (Figs 44, 45). In a 42-day-old embryo the neuropile of the cups of the mushroom body becomes glomerular, i.e. synapses form in this region. These glomeruli become more distinct after 50 days of embryonic development.

(5) The central body (corpus centrale) (Figures 44, 45)

The central body, a neuropile structure, owes its name to its central position in the brain (in the protocerebrum). The rudimentary central body is first seen in the 11-day-old embryo brain. It consists of a distinct mass of nerve fibres in the centre of the main mass of neuropile of the protocerebrum. In the 30-day embryos the central body becomes more distinct and is differentiated into a segmented upper part (with 8 segments) and an unsegmented lower part. This lower part also becomes segmented into eight compartments by the forty-second day. The two ventral tubercles of the central body also appear for the first time in the brain of the 42-day embryo.

(6) The deuto- and tritocerebrum

The deutocerebrum and tritocerebrum are formed, like the protocerebrum, by the activity of neuroblasts and by the production of axons from the ganglion cells

Figure 46. Photomicrograph of a frontal section through the brain of a 42-day-old embryo of P. americana, showing deutocerebral commissure (DTC) or transverse commissure.

Figure 47. Photomicrograph of a frontal section through the brain of a 42-day embryo of P. americana, showing 1st suboesophageal commissure (ISOC).

Figure 48. Photomicrograph of a following frontal section through the brain of a 42-day embryo, showing 2nd suboesophageal commissure (II SOC), circum-oesophageal connective (COESC), and the suboesophageal ganglion (SOG).

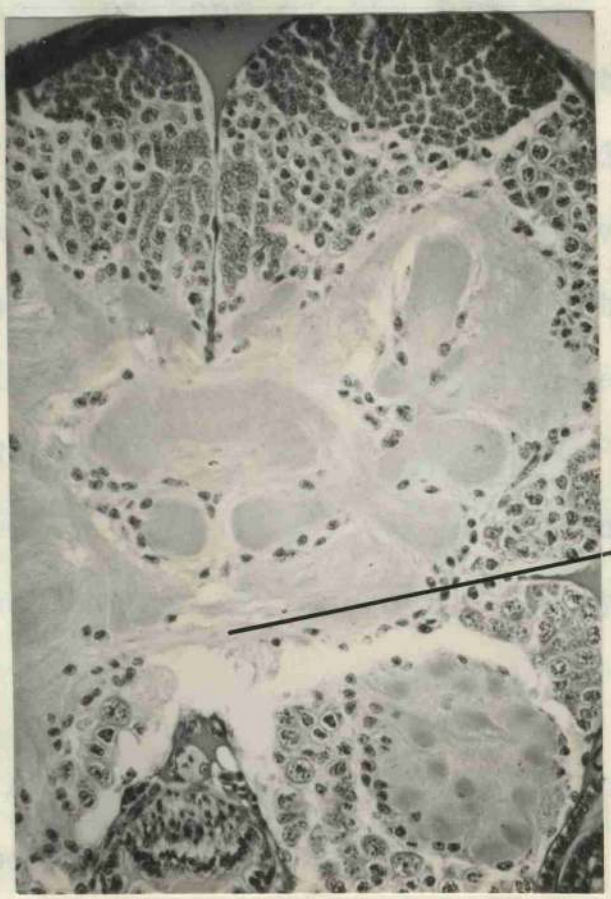


Figure 46.

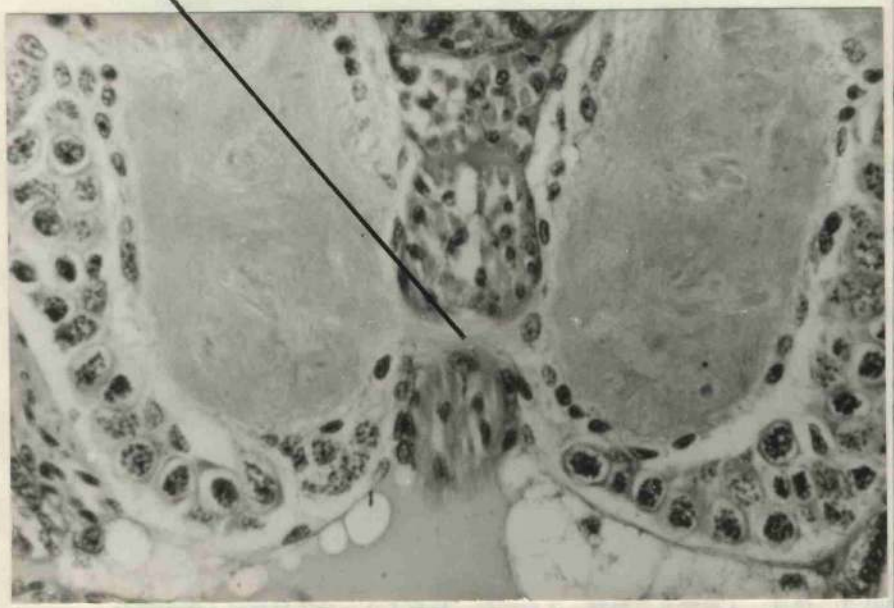


Figure 47.

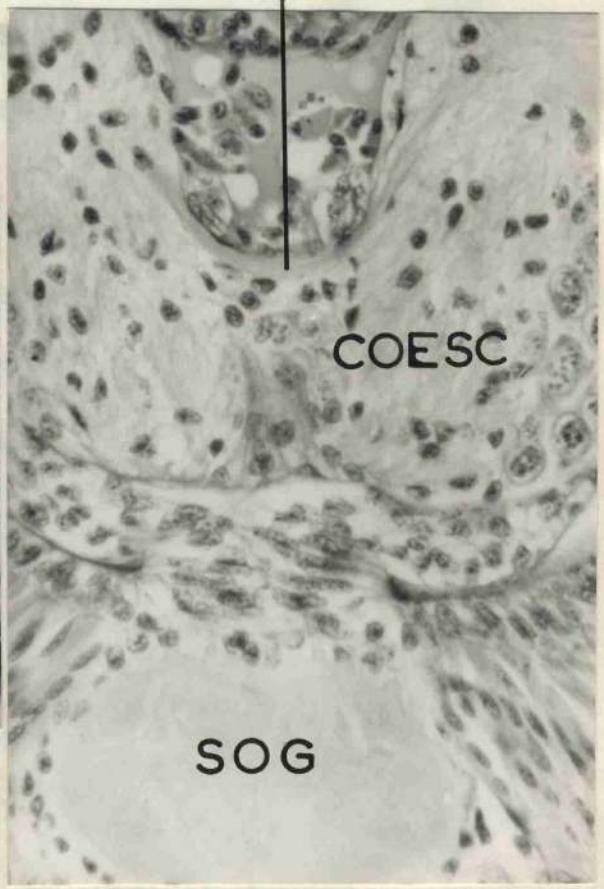


Figure 48.

of these regions of the brain. The deutocerebrum lies anterior to the stomodaeum but the tritocerebrum is post oral in position in the older embryos, nymphs, and adults. The antennary nerve arises from the deutocerebrum. This agrees with the observations of Viallanes (1891) on Mantis, Wheeler (1893) on Xiphidium, and Roonwal (1937) on Locusta. The neuropile of the deutocerebrum, which is simple up to the thirty-fifth day becomes glomerular in 42-day embryos (Fig. 45) as does that of the tritocerebrum.

A clear line demarcating the protocerebrum and the deutocerebrum, is first seen in 20-day embryos. However, even before the appearance of this clear line these two parts of the brain are easily distinguished from one another by their relative position.

The neuropile of the deutocerebrum of each half of the embryonic brain could be seen to be connected to its partner by a distinct neuropile band or deutocerebral commissure. This was reported by Viallanes (1891) in Mantis but not detected in Locusta by Roonwal (1937) who appeared to doubt the former's observation. This commissure lies just ventral to the protocerebral neuropile and is intracerebral. The two lobes of the tritocerebrum are connected by two neuropile bands,

the suboesophageal commissure which are extra-cerebral and pass under the oesophagus. The intra-cerebral supraoesophageal commissure, protocerebral or pons cerebralis, which lies transversely and dorsal to the foregut, arises from the protocerebrum where as the suboesophageal commissures arise from the tritocerebrum. These observations on the origin of supra- and suboeso-:phageal commissures are in agreement with those of Roonwal (1937), but disagree with those of Heymons (1895), Strindberg (1913) and Nelson (1915). The circum-oesophageal connectives are formed from the tritocerebrum and unite it to the suboesophageal ganglion. A nerve (NCCIII, see Chapter 1) arises from the anterior inner lateral surface of each tritocerebral lobe and innervates the corpus cardiacum. A tegumentary nerve (see Chapter 1) originates from the antero-dorsal surface of each tritocerebral lobe and goes to the dorsal part of the head. The tritocerebrum also innervates the labrum and is connected with the frontal ganglion by frontal connectives.

Section III: The Suboesophageal Ganglion

The paired ganglia of the madibular, first maxillary and second maxillary (labial) segments form separately in the early embryo but before eclosion they

unite to form the single suboesophageal ganglion. The neuroblasts which take part in the formation of the suboesophageal ganglion are, as might be expected, identical with those in the embryonic brain. All the nerves of the suboesophageal ganglion mentioned in Chapter 1 could be seen in the older embryos.

Section IV: The Suboesophageal Bodies

In P. americana, the suboesophageal bodies were first seen in the posterior sections of the head of 10-day-old embryos, extending anteriorly almost as far as the brain and posteriorly into the prothorax. It is generally accepted that they are mesodermal in origin. Their cells contain neither globules nor vacuoles in 10 and 11-day embryos, but in 20 (Fig. 49) and 25-day embryos they are full of globules of various sizes some of which stain with paraldehyde fuchsin while others take the counterstain indigo carmine. The cells of this paired structure are either uninucleate or binucleate. The nuclei of the cells of the suboesophageal bodies of 20 and 25-day embryos are clearly bigger than those of either 10 and 11-day or 30 and 35-day embryos. At 30 and 35 days the cells appear vacuolated and possess only a few small green globules. This may be an indication that these cells are at the peak of their activity at

Figure 49. Photomicrograph of a section through the suboesophageal body. Gl, globules; N, nucleus.

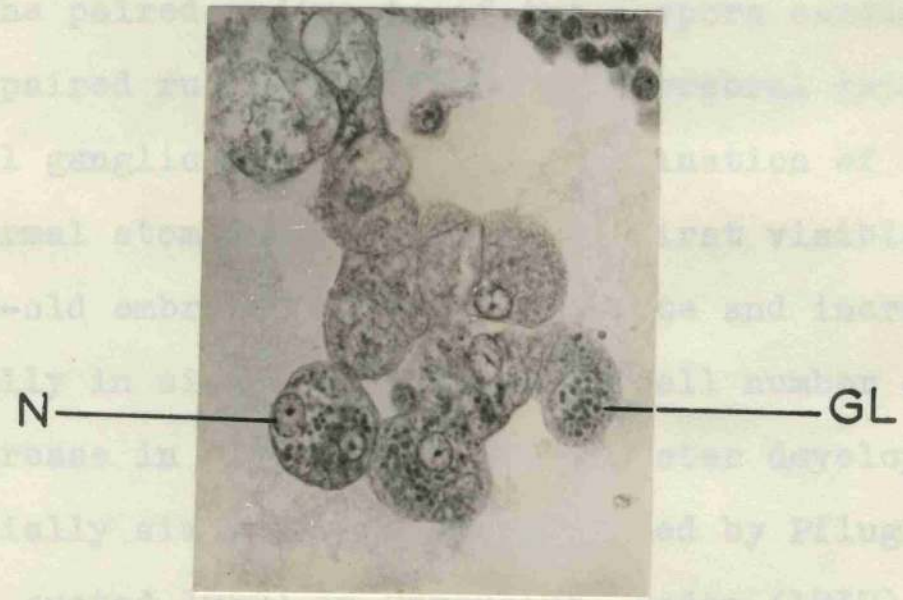


Figure 49.

the twentieth to twenty-fifth day of embryonic development. These bodies disappear after 35 days and seem, therefore, to be organs of the earlier embryonic stage only.

Section V: Origin and Development of the Stomatogastric Nervous System and Retrocerebral Complex

The paired rudiments of the corpora cardiaca, and the unpaired rudiments of the hypocerebral ganglion and frontal ganglion arise from an evagination of the ectodermal stomodaeum. They are first visible in 10-day-old embryos, and differentiate and increase gradually in size, by increase in cell number accompanied by increase in cell size. Their later development is essentially similar to that described by Pflugfelder (1937, quoted 1952) in Carausius, Baden (1937) in Melanoplus, and Roonwal (1937) in Locusta. In P. americana, the paired corpora cardiaca assume their characteristic shape, and final position (i.e. dorsal to the gut, near the dorsal aorta, close to and on either side of the recurrent nerve which arises from the posterior end of the frontal ganglion) in 30-day and later embryos (Figs 50, 51).

Several types of cells can be recognised in these glands in 30-day and older embryos. These are:

Figure 50. Photomicrograph of the frontal section of the corpora cardiaca of a 42-day embryo of P. americana, containing neurosecretory granules. CC, corpora cardiaca; RN, recurrent nerve; Gr, PF-positive neuro-:secretory granules; Ao aorta, Oes, oesophagus.

Figure 51. Photomicrograph of the frontal section of the corpora cardiaca of a 42-day embryo of P. americana, containing neurosecretory granules. This section is a succeeding section shown above in Figure 50. Lettering as in Figure 50.

Figure 52. Photomicrograph of the frontal section of the corpora allata (CA) of a 50-day embryo of P. americana. HG, hypocerebral ganglion; DVC, dividing cell; other lettering as in Figure 50.

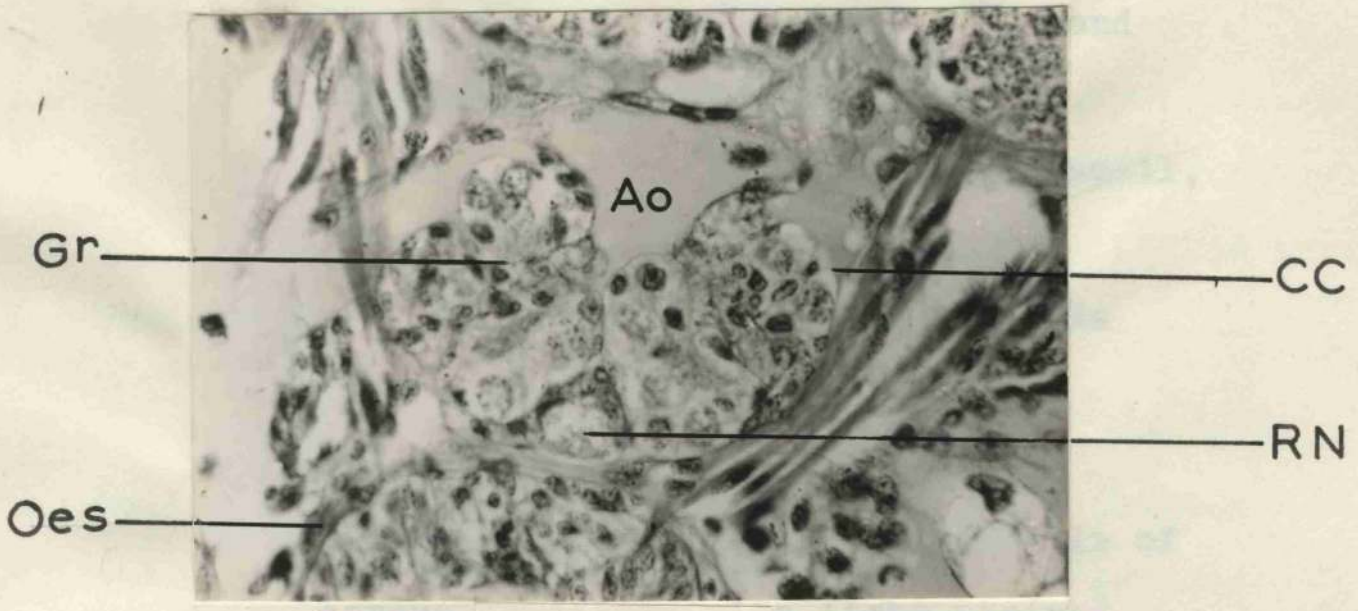


Figure 50.

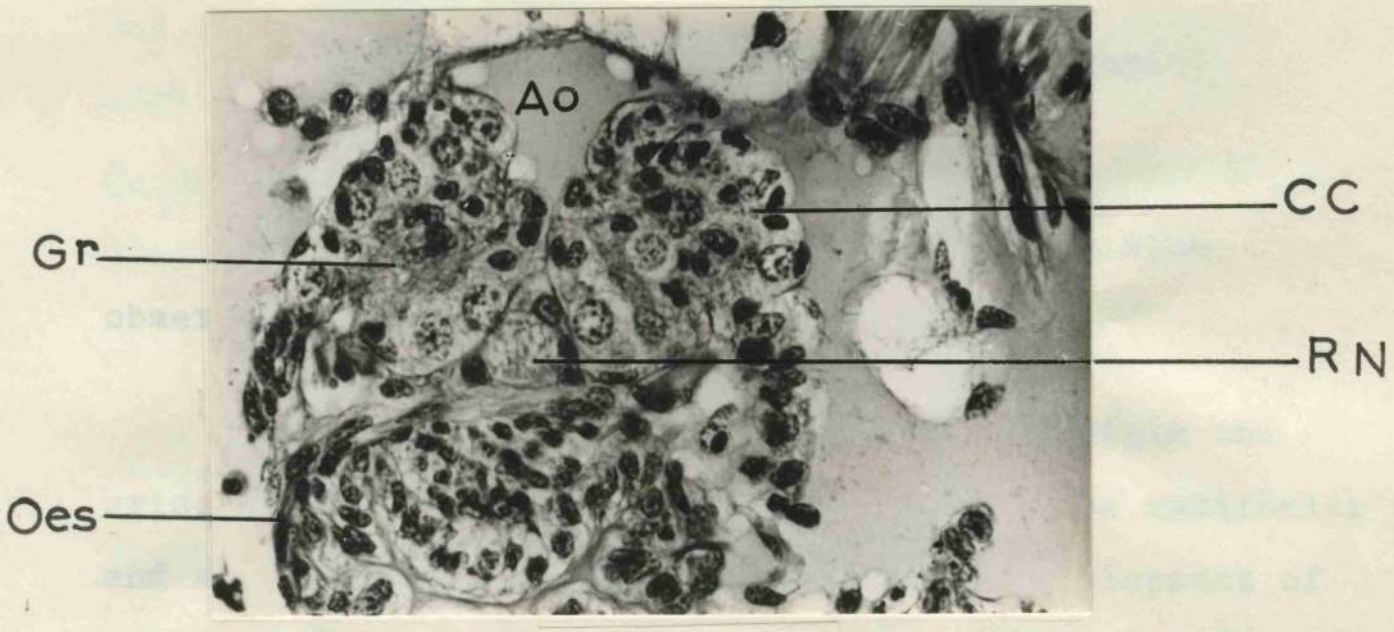


Figure 51.

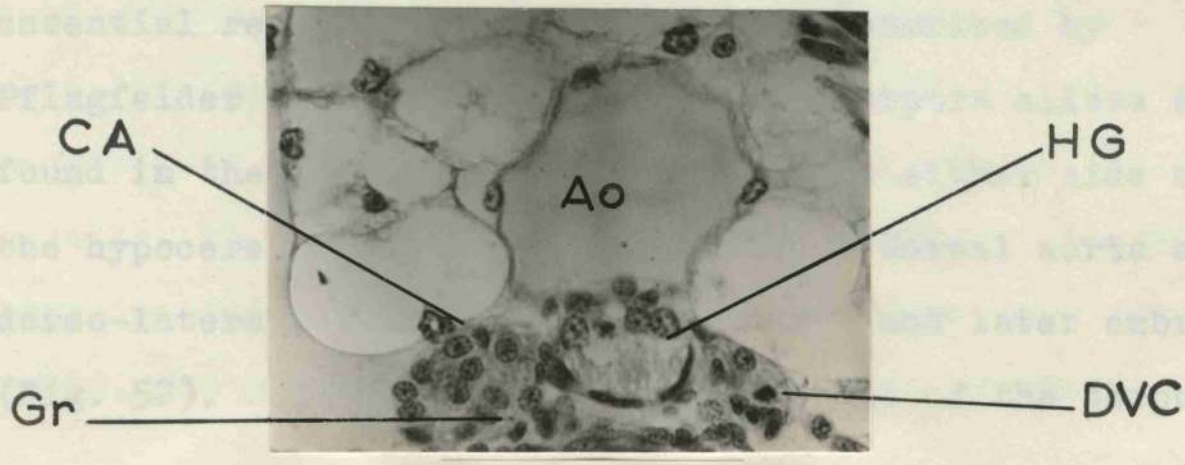


Figure 52.

(a) the perineurium cells, with dark and more or less elongated nuclei;

(b) interaxonal glia cells which are small, round or oval with dark nuclei;

(c) smaller round or subspherical cells of corpus cardiacum with darker nuclei;

(d) larger round or slightly oval cells of corpus cardiacum with light stained nuclei and

(e) small, elongated, darkly stained nuclei of tracheal cells.

Pycnotic nuclei and dividing cells were also observed in the developing corpora cardiaca.

The corpora allata are ectodermal in origin and arise as invaginations at the junction of the mandibular and maxillary segments. The embryonic development of the corpora allata in P. americana resembles in all essential respects that of Carausius described by Pflugfelder (1937, 1952). The oval corpora allata are found in their normal position lying on either side of the hypocerebral ganglion close to the dorsal aorta and dorso-lateral to the foregut in 30-day and later embryos (Fig. 52). From now on the development of the corpora

allata is gradual and continuous throughout later embryonic and nymphal life. Dividing cells are present in the corpora allata of early embryos and occasionally in later embryos and nymphs when the corpora allata have assumed their definitive position.

Larger and smaller cells of corpus allatum, glia cells and perineurium cells as described in Chapter 1 were also observed in the embryonic CA. A nervous connection between the CA and the suboesophageal ganglion and the commissure of the CA connecting the two CA together, undescribed as yet in the embryos of P. americana, were seen in older embryos and are noted here for the first time.

DISCUSSION

Flask-shaped differentiating neuroblasts of the type described by Poulson (1950) in the early embryonic brain of Drosophila can be seen, up to the tenth day of development, in the embryo of P. americana. It is certain that the smaller cells produced by unequal division of neuroblasts, i.e. ganglion mother cells, divide again, equally, to form daughter cells which normally become ganglion cells although daughter cells sometimes divide again, the products of this last division becoming

ganglion cells. There is no evidence, at least in P. americana, to support Wheeler's (1893) theory that the smaller cells produced by the division of neuroblasts become ganglion cells.

It is also certain that neuroblasts do take part in the formation of the of the optic lobes. Pflugfelder (personal communication) has concurred with the author's finding on this point. The elongated flask-shaped cells in the early embryonic optic lobe of Xiphidium which Wheeler (1893) distinguished, on the basis of morphology, from the neuroblasts can be found in early embryonic stages of P. americana, but it subsequently becomes clear that these are simply neuroblasts at an early stage of development. It is difficult to understand why some of the earlier authorities were unwilling to recognise the occurrence of neuroblasts in the optic lobe. Not only neuroblasts but also the young ganglion cells of the optic lobe are similar to those of the other protocerebral lobes in a 10-day embryo. These neuroblasts occur at the periphery of the optic lobe and in the lamina ganglionaris and intraganglionic thickening. The peripheral neuroblasts disappear during later embryonic development but neuroblasts found in the other locations persist until the last nymphal instar.

The invagination of the dermatogenic cells overlying

the primordium of the intraganglionic thickening may signify the incipient separation of the first and second protocerebral lobes. This invagination carries the neuroblasts of the intraganglionic thickening inwards. By the twentieth day the thickening has separated from the hypodermis and its lower part has extended inwards and lies between the medulla externa and medulla interna. The complete transformation of the outer neuroblasts, by repeated divisions to form ganglion cells, results in the disappearance of the outer part of this structure, the inner region persisting as noted above. This change was wrongly interpreted by Viallanes (1890) and Wheeler (1893) as a migration. Baden (1937) appears to have missed the intraganglionic thickening in early embryos of Melanoplus, and to have failed to detect the relationship between the neuroblasts adjacent to the hypodermal indentation and the structure lying deep in the brain at a later stage in embryonic development. There is no second intraganglionic thickening, as suggested by Strinberg (1913), in P. americana but in the 42-day embryo the intraganglionic thickening extends in the form of a narrow horseshoe-shaped band of meristematic cells as far as the third protocerebral lobe. This structure, which consists of small neuroblasts and still smaller ganglion mother cells, is not observable in

later embryos.

The author's description of the neuropile masses of the optic ganglion, chiasmata and optic nerve agrees with that given by Viallanes (1891), Bauer (1904) and Roonwal (1937). The failure of Baden (1937) to detect some of these structures may be due to the fact, noted earlier, that they are very small, appear on a few sections only and can easily be missed.

In the 20-day embryo of P. americana some of the perineurium cells bear a superficial resemblance to some of the overlying dermatogenic cells which are destined to form the head capsule but this is meagre evidence on which to base a theory, as attempted by Heymons (1895), that the former arise from the latter. This theory was discounted by Strindberg's (1913) observation that in specimens of three different orders the perineurium is formed some time after the brain has separated from the hypodermis. There is no evidence, in sections of P. americana embryos, to support the idea (Nusbaum, 1883; Wheeler, 1893) that perineurium cells are derived from median cord elements. As has been stated earlier, a number of workers have suggested that the perineurium cells are modified peripheral ganglion cells which are the outer products of peripheral neuroblasts. However, the nuclei of the perineurium cells are much smaller

than those of the ganglion cells, are usually elliptical in shape whereas the latter are usually round, and the nuclei of the former stain more intensely with Groat's haematoxylin than those of the latter. Moreover, when they are first identifiable the perineurium cells lie outside the peripheral neuroblasts. When these neuroblasts divide the products of division usually lie to the inside. Perineurium cells can only be derived from these neuroblasts if they have (a) migrated to the outside and (b) undergone a rapid and drastic differentiation. There is no histological evidence to support either possibility. On the other hand, the similarity between perineurium cells and glia cells, which has been emphasised earlier, provides an important clue to the solution of this problem.

Strindberg (1913) and Pflugfelder (personal communication) have supported the theory that glia cells are modified ganglion cells and are thus ectodermal in origin although the latter ascribes a separate mesodermal origin for the perineurium.

Only two workers in the past recognised the fundamental importance of immigrant cells. Korotneff (1885) described the invasion of the developing nervous

system of Gryllotalpa by wandering blood cells which, he thought, formed the structure now called the perineurium, while Bauer (1903, 1904) described a similar invasion of the developing nervous system in the prepupa of Culex by connective tissue cells which become glia cells. Each was to some degree correct since the evidence now presented indicates that perineurium cells and glia cells are derived from cells of mesodermal origin, principally those of the cephalic coelomic rudiments, which actually invade the developing brain.

All theories concerning the origin of perineurium and glia cells which (a) suggest that either are ecto-:dermal in origin, (b) suggest that either are derived from ganglion cells and (c) suggest that they have a dissimilar origin, are now rejected. This conclusion supports the view of Wigglesworth (1959, 1960) that the perineurium cells have probably been derived from glia cells which have become specialised for protective, nutritive and excretory functions.

Regarding the origin of the neural lamella there is no substantial evidence that haemocytes play a part in its formation in P. americana. The anastomosing protoplasmic processes, of the dermatogenic cells which

have separated from the brain, certainly terminate on the neural lamella in 20 and 25-day embryos. In the 30-day embryo the dermatogenic cells have lost their connection with the neural lamella. It is true that from the study of sections of earlier embryos one might think that the dermatogenic cell processes produce the neural lamella but a study of later sections makes it clear that the protoplasmic extensions simply represent the final stage in the separation of the dermatogenic cells from the brain. It seems fairly certain that the perineurium cells with which the neural lamella is so intimately associated secrete this outer protec-:tive layer.

There is no doubt that the supraoesophageal commissure which unites the two halves of the protocere-:brum is formed by the axons of neurons lying in the protocerebrum and so the possibility that it is produced from a 'median ectodermal thickening' (Heymons, 1895) does not arise. The two lobes of the tritocerebrum are connected by two suboesophageal commissures, both of which are formed by axons of neurons in the tritocerebrum. It has long been known that insects possess a suboesophageal commissure but recently Willey (1961) stated that some insects possess two

such connections. The presence and origin of two trito-:cerebral commissures in an insect embryo is recorded here for the first time.

It has variously been suggested that the suboesopha-:geal bodies are either excretory or blood forming organs. Wheeler (1893) who initiated the former theory stated that the nuclei of the cells of these bodies remain constant in size but this is not so in P. americana in which they increase in size from the tenth to twenty-fifth day and thereafter decline. Since mitoses were not observed in these bodies subsequent to their formation in the embryo, it seems improbable that they could be the sites of production of blood cells. Their function must for the moment remain in doubt.

Finally, these investigations support those of all the other workers who have ascribed an ectodermal origin to the corpora allata. The observation of dividing cells in the CA of later embryos and nymphs contradicts the reports by Wiesmann (1926) and Pflugfelder (1948) that cell division in the CA of Apis occurs only up to the time of its detachment from the ectoderm; but such dividing cells have been reported in the CA of many other insects (Pflugfelder, 1952).

CHAPTER 3

NEUROSECRETION IN THE EMBRYONIC STAGES AND LATER STAGES IN THE DEVELOPMENT OF THE COCKROACHES, PERIPLANETA AMERICANA L., AND BLATTA ORIENTALIS L.

INTRODUCTION

(1) Hormones in insects

The first evidence that insect metamorphosis is under endocrine control was provided by the experiments of Kopec (1917, 1922) with larvae of Lymantria dispar L. These involved firstly, the division by ligature of the bodies of last instar larvae. If pupation occurred shortly after ligaturing then both halves of the body were affected but if it occurred some time after ligaturing then only the anterior half of the body was involved. The second series of experiments involved the extirpation of the most obvious possible controlling centre in the anterior part of the body, namely the brain. The results again depended on the relationship between the time at which extirpation was performed and the time at which pupation was due to occur. If extirpation was performed shortly before pupation then the animal still pupated, but if the operation was performed some time earlier then it

remained in the larval state.

Subsequently a number of other workers, notably Fraenkel (1935), Caspari & Plagge (1935), Kühn & Piepho (1936), and Plagge (1938), performed similar ligaturing experiments on last instar larvae of Diptera and Lepidoptera with similar results. Meanwhile, Wigglesworth (1933, 1934, 1936) by decapitation and parabiotic experiments on the bug, Rhodnius, produced conclusive evidence that there is a hormone which causes moulting in insects. There was not at that time sufficient histological evidence to indicate that the brain of insects acts as an endocrine gland. On the other hand, Nabert (1913) had described the corpora allata (CA) in insects from a number of orders and had pointed out that the histological appearance of these bodies indicated that they were, in fact, endocrine glands. It was perhaps reasonable, therefore, to propose that the source of moulting hormone was not the brain but was the adjacent CA (Wigglesworth, 1934, 1936; Handorn, 1937; Handorn & Neel, 1938; Burtt, 1938).

The first example of neurosecretory cells (NSC) in an insect was provided by Weyer (1935) in Apis.

Scharrer (1937) found gland-like nerve cells in Bombus, whilst neurosecretory cells were demonstrated in the dorsal part of the brain of Rhodnius by Hanström (1938). Working from Hanström's evidence, Wigglesworth (1939, 1940) was able to show in a decisive way by extirpation experiments with Rhodnius that the source of the hormone controlling moulting lay in the dorsal median part of the brain. If the NSC demonstrated by Hanström (1938) were removed from the brain of a Rhodnius larva after a critical period, i.e. after the time when decapitation can prevent moulting, and implanted into a larva decapitated before the critical period, the implants induced moulting in the latter.

Wigglesworth (1940) now showed that the CA produce not the moulting hormone but another hormone, subsequently named neotenin or juvenile hormone, whose function is to restrain the development of imaginal characters at larval moults. Due to the work of E. Scharrer and B. Scharrer (1944), B. Scharrer (1946c, 1951, 1952a,b,c), and E. Thomsen (1954) it became evident that the corpora cardiaca (CC) of insects act as organs of storage and release of neurosecretory material outside the brain and that the neurosecretory material is transported along the axons of the brain

NSC, which form the nerves to the CC. Fukuda (1940a,b, 1944) discovered that in Bombyx the prothoracic glands and not the brain secrete moulting hormone.

Williams (1946, 1947, 1948a, 1952) reconciled this apparent disagreement by showing in Hyalophora (Platysamia) that the brain releases a trophic hormone which stimulates the prothoracic glands which in turn produce moulting hormone. It is the prothoracic gland hormone (moulting hormone or ecdyson) which acts on the tissues of the body to produce the changes characterising the moult. This has proved to be the case, not only in Hyalophora, but also in Rhodnius (Wigglesworth, 1952a, b), in Orthoptera (Pflugfelder, 1947), in Odonata (Deroux-Stralla, 1948), in Diptera (Possompes, 1950, 1953) and in numerous other insects which have been investigated since.

Prothoracic glands of the higher pterygote insects are equivalent to the ventral glands of lower Pterygota (Pflugfelder, 1952), to the thoracic glands of Hemiptera (Wigglesworth, 1952a), and to the peritracheal gland or lateral ring-gland cells of dipteran larvae (Possompes, 1953).

Prothoracic glands in Orthoptera were first

described by Scharrer (1948a) in the cockroach, Leucophaea, these she considered homologous with the prothoracic glands of Lepidoptera. Bodenstein (1951) from his extirpation experiments concluded that the prothoracic glands are essential for the process of moulting and metamorphosis in Periplaneta. In adult Periplaneta the prothoracic glands degenerate completely some 12 to 14 days after metamorphosis (Bodenstein, 1953b). Thoracic glands break down and disappear within 48 hours after final moulting in Rhodnius (Wigglesworth, 1951). In all normal adult pterygote insects belonging to different orders the prothoracic glands or their equivalent degenerate after the final moult, so that no further moult occurs in the adult insects.

Exhaustive reviews on neurosecretion in insects have been produced by Pflugfelder (1941, 1952), E. Scharrer & B. Scharrer (1945, 1954a, 1954b), B. Scharrer (1948b, 1952a, 1953a), Wigglesworth (1951, 1954a, 1957a), Gabe (1954), Nayar (1955b), Johansson (1958), and most recently by Van der Kloot (1960). Neurosecretion in insects has also been discussed at the three international symposia on neurosecretion - Naples (1953), Lund (1957) and Bristol (1961).

An NSC is a special type of nerve cell. It shows

pronounced glandular activity. In appropriately stained sections it is seen to contain a particulate product. This product stains blue-black after chrome haematoxylin phloxin staining, and pink to purple-red after paraldehyde fuchsin, Groat's haematoxylin and indigo car-:mine staining procedure, and may be traced in the axon. An NSC resembles the ordinary neurons in that it receives nerve impulses and conducts them, but it differs from the ordinary nerve cells in that it does not pass the impulses to other nerve cells or to effector organs such as mucles; instead its axon ends against the wall of the blood vessel (in insects) or sinuses (in crustaceans) into which the hormones produced by the cell are discharged.

NSC have been described in many insects. In the protocerebrum of the pterygote insect brain two groups of NSC, namely the median neurosecretory cells (MNSC) and the lateral neurosecretory cells (LNSC), are known to occur. The MNSC lie close to the pars intercerebralis. Their axons form the nervi corporis cardiaci interni (= NCCI, Pflugfelder, 1936-37; Hanström, 1940). The axons of many of the NCCI of one side cross over to the other half of the pars intercerebralis and finally leave the brain from its ventral surface to enter the CC.

The LNSC may lie either in the pars intercerebralis or more usually in the lateral part of the protocerebrum. The axons of the LNSC pass directly to the rear of the brain to form the nervi corporis cardiaci externi (NCCII, Hanström, 1940) which after leaving the brain enter the CC.

MNSC and LNSC are activated at some time during an instar and a substance is produced in the perikaryon of these cells. This is loosely described as neurosecretory material and occurs in the form of minute granules which may have a definite structure, the active component being enclosed in a capsule of a different substance (E. Scharrer, personal communication). According to E. Scharrer & S. Brown (1961) the formation of neurosecre-:tory granules in Lumbricus at least, is completed by the Golgi apparatus. Substances produced by the NSC of the protocerebrum are said to perform at least the following six functions:

1) activation of the prothoracic glands,
2) stimulation of protein synthesis in the gut (E. Thomsen & Møller, 1959),
3) control of water retention (Nayar, 1960),
4) control of egg development and

oviposition,

5) control of the activity of the CC and CA, and

6) control of activity rhythms (Ozbas & Hodson, 1958).

Neurosecretory material from the protocerebral NSC is transmitted via the NCCI and NCCII to the CC (E. Scharrer & B. Scharrer, 1944; B. Scharrer, 1951, 1952a, b,c; E. Thomsen, 1954), and from the tritocerebral NSC also to the CC via the NCCIII (Dupont-Raabe, 1956, 1957, 1958). According to Dupont-Raabe the hormone produced by the NSC of the tritocerebrum exerts an endocrine control over the diurnal colour changes of Carausius.

After a short sojourn in the CC the neurosecretory material is released into the blood in the lumen of the aorta. Thus the CC of insects are, by the definition of Carlisle & Knowles (1953), neurochaemal organs in which nerve terminations are in contact with a blood vessel or a blood sinus. The neurosecretory material does not actually enter the cells of the CC (Stutinsky, 1952; Hanström, 1953). M. Thomsen (1954) concluded that the axons of the NCC diverge and branch in the CC and the neurosecretory material is stored in the termini of these axons. These termini appear as swollen

structures filled with the stainable neurosecretory product. These structures resemble the herring bodies found in the vertebrate nervous system.

Little is known about the mechanism of controlling the discharge of neurosecretory material from the CC. Johansson (1958) has suggested that it may be controlled by nerve impulses coming from neurons other than the NSC. According to Hodgson & Geldiay (1959) neurosecretory material is released from the CC of Blaberus when the animal is hyperactive or is under conditions which produce a symptom of stress, e.g. if electric shocks are applied.

Grandori (1954, 1955) has described in Musca and Calliphora, a capillary system surrounding the nerve cells of the brain and the cells of the CC. According to her these capillaries are in close contact with the cardiac nerve fibres and the tracheal system. They are said to be derived from subdivisions of haemolymphatic vessels. This capillary system starts in the anterior region of the brain and is a kind of drainage system whose function is the transport of neurosecretory material towards the CC. As far as is known Grandori is the only author who describes in insects such a method of transportation of neurosecretory material. She states that neurosecretory material from the axons of the NCC

in the CC and the material secreted by the CC itself are given off into the aorta by these fine capillaries.

In crustaceans, a capillary system of this type is known (Matsumoto, 1954, 1956, 1958), but in insects its existence is not well established.

It is not yet certain whether the CC modify the neurosecretory product of the brain cells or are merely storage and release organs; but according to Possompes (1953), Vannucci (1953), and others, their intervention is essential.

The potency of isolated active brain implants in Platysamia (Williams, 1948b) might suggest that the CC is not essential but Stum-Zollinger (1957) has shown that in this insect the severed fibres of the NCCI and NCCII regenerate structures analogous to the CC, and in these substitute organs the neurosecretory materials from median and lateral neurosecretory cells come together and are stored. In addition to their storage and release functions the CC may also act as organs of internal secretion. As described by Cazal (1948) there are two types of cell in the CC, the smaller cells, which he described as chromophobe cells, which are usually regarded as the glia cells; and the large chromophil cells. It seems reasonable to suppose that the large

cells are the secretory cells. In an electron microscope study of the CC of cockroaches, Willey & Chapman (1960) have described within the large cells secretory granules up to 6000 Å in diameter.

Effects of the CC extract on the heart and other organs have been described by Cameron (1953), Vannucci (1953), Wigglesworth (1954b), Herlant-Meewis (1956) and others. It might be argued that the effect produced by CC extract is not due to a secretion of these organs but to neurosecretory material which has been stored within them. However, Cameron (1953) suggested that the heart-activating substance, an orthodiphenol (but not adrenaline) does not come from the brain but from the CC. Phloxinophil secretory material has been recorded in the CC in Ephemeroptera (Arvy & Gabe, 1952a), Odonata (Arvy & Gabe, 1952b), Thysanura (Gabe, 1953b), Bombyx (Arvy, Bounhoil & Gabe, 1953), Plecoptera (Arvy & Gabe, 1954a,b), Iphita (Nayar, 1956), Anisolabis (Ozeki, 1958b), Periplaneta (Füller, 1960) and Schistocerca (Highnam, 1961), but no serious attempt has been made to equate this with the adrenaline analogue.

Neurosecretory material containing prothoracotrophic hormone is carried away from the CC in the blood stream, and when this hormone reaches a threshold titre in the

blood the activity of the prothoracic glands is established. These glands produce the moulting hormone or ecdysone as noted above, and when in turn ecdysone reaches a threshold concentration it renders inactive the brain cells responsible for its evocation (Williams, 1947). It is not yet certain whether the brain NSC in the premoult larva are concerned only with the activation of the prothoracic glands. Kobayashi & Burdette (1961) state that pupation cannot be induced in the isolated larval abdomen of Calliphora by injection of the brain hormone alone or by injection of ecdysone alone but only by the combination of both hormones in certain proportions. They state, in fact, that both the ecdyson and the prothoraco-:trophic hormone are required to bring about pupation and that the brain hormone, in addition to activating the prothoracic glands, may also act directly on the tissues of the body. A conclusion which perhaps implies that the only hormone produced by brain cells is prothoraco-:trophic hormone which might also act on the body tissues. Fraser (1957), however, having separated the NSC in the larva of Lucilia into six groups on criteria of position, size, staining characteristics, time and duration of activity, claimed that it was probable that no more than three of these groups were concerned with thoracic gland activation, though all function during metamorphosis;

and he suggested that the other NSC groups must be concerned in the control of other physiological processes associated with moulting and metamorphosis. It is not therefore necessarily prothoracotrophic hormone, but possibly another brain hormone which is required to act directly on the tissues along with ecdyson.

Chadwick (1955, 1956) claimed from the results of his extirpation experiments with Periplaneta nymphs, that moulting can take place even after the removal of the prothoracic glands. It would be safe to state that complete removal of the prothoracic glands is very difficult if not impossible, and the conclusion of all other investigators in the field of insect endocrinology - that the prothoracic glands produce the hormone ecdyson, which induces moulting - is accepted.

Recent observations on insect endocrine organs and neuro-:secretion

The various organs of internal secretion are in constant interaction with each other (Bodenstein, 1951, 1954). Until recently all the experimental evidence available supported the view that the outcome of a moult depends on the relative concentrations of ecdyson and juvenile hormone (neotenin) present in the blood of the

insect at the time of the moult, and also on the degree of responsiveness of epidermal tissues towards these hormones (Bodenstein, 1954). In nymphal or larval stages a higher concentration of juvenile hormone and a lower concentration of ecdyson in the blood lead to a moult without metamorphosis. But at the last moult in Exopterygota or at the penultimate moult in Endopterygota there appears to be a shift in the balance between the ecdyson and juvenile hormone in favour of the former. As a result of this the juvenile hormone can no longer exert its restraining influence on adult differentiation and thus metamorphosis occurs (Scharrer, 1946a; Bodenstein, 1951, 1954; and others). Recently, however, Halbwachs et al. (1957), have shown that it is possible to achieve a partial inhibition of differentiation of wing lobes and genital apparatus by implanting extra ventral glands (prothoracic glands) into moulting nymphs of Locusta, i.e. precocious augmentation of the concentration of ecdyson inhibits differentiation. Wigglesworth (1961) has shown that the application of either pure juvenile hormone or of farnesol (which produces all the known effects of corpus allatum hormone on nymphs and adult females of Rhodnius) to the cuticle of fourth instar Rhodnius nymphs does not result in the restraining of differentiation of the wing lobes and external genital apparatus after the

next moult. In other words, neither juvenile hormone nor its equivalent farnesol seems to have any inhibitory influence on the differentiation of these structures. Wigglesworth (1961) agrees with Halbwachs et al. (1957) in concluding that the progressive increase in size of the wing lobes and progressive differentiation of the external genital apparatus during nymphal life is not under the control of juvenile hormone, though its presence is essential to prevent precocious metamorphosis. Thus according to Wigglesworth (1961) two distinct kinds of differentiation can be distinguished during the post-embronic life of exopterygote insects. In the first, represented by the progressive differentiation of such ectodermal structures as wing lobes, and genital appendages at nymphal moults, the degree of differentiation depends on the concentration of ecdyson and on the time at which a critical concentration of ecdyson is achieved. If the concentration of ecdyson reaches a high level in a short time, the cuticle will be laid down quickly and therefore differentiation will be limited. Delay in cuticle formation results in a greater degree of differentiation. The second kind of differentiation refers to the radical changes collectively known as metamorphosis.

Metamorphosis is under the control of juvenile hormone.

According to Wigglesworth (1957a, 1961) the genes which are responsible for the formation of the larval type of cuticle are 'kept in action' by juvenile hormone, but at the last larval or nymphal moult - in the absence of juvenile hormone - those genes which are responsible for producing the adult type of cuticle and various other adult structures can now express themselves, while the former genes no longer exert their influence. It is interesting to note that Ozeki (1958a) observed that the development of male external genitalia in the earwig, Anisolabis, is not under the control of juvenile hormone but that juvenile hormone controls the development of internal reproductive organs.

Not all the axons of the NCC terminate in the CC but some of them pass through it to enter the CA on the same side. These form the nerve called nervus corporis allati (NCAI) as first described by Hanström (1940). There is another nerve (NCAII) (see Chapter 1) connecting the CA with the suboesophageal ganglion (SOG). This nerve has been called the caudal nerve by Füller (1960). Although according to Bodenstein (1954) the causes of activation of the CA are uncertain, there is at least a fairly general agreement that the CA act under the influence of brain NSC, The neurosecretory material

from the NSC of the protocerebrum is transported to the CA via the NCAI (Stutinsky, 1952; Arvy, Bounhoil & Gabe, 1953; Arvy & Gabe, 1953a,b,c, 1954b; Bounhoil, Gabe & Arvy, 1953, 1954; M. Thomsen, 1954a,b; E. Thomsen, 1954; Nayar, 1956, and others).

According to Scharrer (1946c, 1952b, 1961) the brain NSC restrain the activity of the CA in Leucophaea. This observation was based on experimental evidence that after severing the connections between the brain and the CA, the latter increase in size, and become active. The first histological evidence to support this theory is presented in this thesis. However, according to another school of thought the neurosecretory material activates the CA. E. Thomsen (1952) thought that the stimulation might not be totally humoral but also partially nervous. Engelmann (1957) has claimed that control of the function of the CA (in Leucophaea) by the brain may either be nervous or through the neurosecretory material of the brain, though he thought the former more likely. He further asserted that the CA are excited by the SOG which is in nervous connection with these glands as noted above. Roth & Stay (1961) have found that mating stimuli activate the adult CA in the female cockroach.

Diploptera

In addition to their role of restraining differentiation at larval moults discussed above, the CA also produce a gonadotrophic hormone in adult female insects (Pfiffer, 1936, 1939; Wigglesworth, 1936, 1948; E. Thomsen, 1940; Scharrer, 1946b; Engelmann, 1960, and others), the secretion of the CA being chemically identical in both cases (Pfeiffer, 1945; Wigglesworth, 1948, 1954a, 1961; Williams, 1959).

The CA of females show cyclic changes connected with reproductive activity (Scharrer, 1946b; Scharrer & Van Harnack, 1961). However, this is not so in the case of the male though the male CA are not completely inactive during the adult life. The ovary in its turn exerts some effects on the activity of the CA since these glands often increase in size in castrated females (Von Harnack & Scharrer, 1956, and others). The fact that the activated ovary restrains the activity of the CA has been interpreted by Johansson (1958) as a typical feed-back mechanism.

In addition to these functions, Engelmann (1959) concludes that the hormone of the CA in the cockroach, Diploptera, activates the accessory glands of the female

genital apparatus and stimulates the secretory cells of the bursa copulatrix - the secretion of which facilitates the extrusion of the spermatophores. In the termite, Kalotermes (Lüscher & Springhetti, 1960) the CA are said to secrete two different hormones which have different effects on the differentiation of castes. In several cockroaches investigated by Barth (1961) the CA of adult females control the production of a volatile sex-attractant (sex pheromones or ectohormones).

Scharrer & Harnack (1958) in Leucophaea, reported secretory granules within the cells of the CA, which stained with paraldehyde fuchsin. They held that this intrinsic secretion was the active principle elaborated in the cytoplasm of the stellate cells of the CA and released into the body fluid surrounding these glands.

(2) Neurosecretion in insect embryos

Neurosecretion during post-embryonic development and in the adult female insect has attracted the attention of many workers but the possibility of, and problems associated with, neurosecretion during embryonic development have scarecely been considered yet. As far as is known, there are available only two contributions to the subject of neurosecretion in orthopteroid insects

during embryogenesis. Jones (1956), in his comparative study of the diapausing eggs of Locustana pardalina (Wlk.) and the non-diapausing eggs of Locusta migratoria L. (for which he used the standard chrome haematoxylin phloxin staining procedure) described large NSC at a very early embryonic stage (the third day after the cessation of diapause in L. pardalina) lying at the periphery of the protocerebrum just below the perilemma. He found that the ventral head glands of L. pardalina and L. migratoria reached their maximal activity on the sixth and eighth days, respectively, of their embryonic development. On the sixth day in L. pardalina and on the eighth day in L. migratoria a moult occurred, the epidermis retracting from the cuticle. From this coincidence and from his ligaturing experiments he concluded that the ventral head glands in locust embryos are responsible for the retraction of the cuticle from the epidermis. He does not mention anything about NSC in later embryonic development. He did not find neuro-:secretory material in the CA and there is no mention of the CC in his work.

Sharan & Sahani (1960) in a study of the 'provisional embryonic cuticles' of Dysdercus cingulatus (Hemiptera) also briefly described large peripheral protocerebral

NSC in the early embryo and, like Jones, found that the CA did not show any cyclic activity and did not receive neurosecretory granules during embryonic development. They too, did not mention the CC.

Füller (1960) studied neurosecretion in embryos, first to third nymphal instars, last nymphal instar, and adult males and females of Periplaneta americana L. He also studied neurosecretion in five nymphal stages and adults of Blatta orientalis L. He used both chrome haematoxylin phloxin and paraldehyde fuchsin stains. He detected the neurosecretory material in the CC of embryos that were ready to hatch within a few hours and of newly hatched nymphs of the first instar but not in neurons of the pars intercerebralis until the second instar. These observa-:tions led him to two possible conclusions, that the CC cells produce the stainable substance themselves or that the endings of the nervi corporis cardiaci (NCC) produce the apparent secretory granules. The latter conclusion applies a neurosecretory hypothesis relating to vertebrates (advanced by Spatz (1953) and his school of thought), to an invertebrate. Füller (1960) did not find LNSC at any stage of development of either P. americana or B. orientalis. He did not describe cyclical changes in the CC or CA not did he find

neurosecretory material in the CA before the fourth nymphal instar.

Though the CC of orthopteran insects have been studied extensively and have been described by many authors, e.g. by Hofer (1887), Police (1910), Nabert (1913), Bretschneider (1914), de Lerma (1933, 1937), Pflugfelder (1937), Cazal & Guerrier (1946), Cazal (1948), Scharrer (1951), Nayar (1954), Füller (1960), Highnam (1961) and others, the presence of neurosecretory granules in the embryonic CC and the cyclical activity of the embryonic CC have not been reported as yet.

NSC were first noted in ventral ganglia (abdominal ganglia) of Lepidoptera by Day (1940). Köpf (1957) found neurosecretory cells in the thoracoabdominal ganglia of Drosophila. According to Scharrer (1941) and Kobayashi (1957) there are more NSC in the SOG of cockroaches and silkmoths respectively than in their brains. Fukuda (1951a,b, 1952, 1953a,b,c) and Hasegawa (1951, 1957) discovered that in Bombyx the SOG produces a diapause hormone, the production of which seems to be under the control of the brain. Harker (1960a,b,c) found that the rhythmic secretion of a hormone from NSC of this ganglion controls the circadian locomotory

activity of P. americana. Although NSC in the SOG of nymphs and adults of P. americana are known to occur (Scharrer, 1941; Drescher, 1960; Füller, 1960) they have not so far been described in the embryo. Due to a shortage of nymphs of P. americana, Scharrer (1941) could not establish with any certainty the stage at which NSC occurred first in the SOG of this species. Drescher (1960) also is silent on this point. As a matter of fact, Drescher (1960) describes only one large cell with a thick tubular axon as NSC situated in the ventro-lateral position in the SOG. Füller (1960) mentions the presence of NSC in this ganglion only in the last instar nymphs and adults.

In view of the lack of detailed information on this subject it was decided that a careful histological study of the central nervous system (CNS) of embryos, nymphs and adults could provide valuable information on such matters as, the stage of development at which NSC are first distinguishable in the CNS, the distribution of NSC of the brain and SOG, the first signs of activity in each NSC group detectable by the ordinary light microscope, cyclical changes in the CC and CA during embryonic and nymphal life of P. americana and during nymphal life in B. orientalis. As far as is known,

no one has presented (in P. americana and B. orientalis) histological evidence of a relationship between the activity of brain NSC and changes in the CC and of CA, as indicated by the changes in the concentration of nuclei, and the nuclear number/cytoplasmic volume ratio in P. americana.

MATERIAL AND METHODS

One hundred and sixteen embryos, nymphs and adults of P. americana of known ages were used in this study. The number of B. orientalis used was 55. After removal of the antennae and mandibles, heads were amputated and fixed in one of the following fixatives: Gilson's, Susa's, Helly's or aqueous Bouin. Of these, aqueous Bouin proved to be the most satisfactory for general purposes, though Helly's gave a better picture of neurosecretory material in the neurons and their axons. The superiority of Helly's as a fixative for neurosecretory material has also been reported by Scharrer (1955b). Usually the tissues were fixed for 12-24 hours.

Eggs of P. americana were incubated at 25°C, development taking 55-56 days at this temperature. A number of batches of P. americana nymphs were reared on dog biscuits and apples and kept in glass jars and small cages from the first instar to imago. Post-

embryonic development took about 13 months, the number of nymphal instars being eleven. The heads of P. americana nymphs of known instars and those of adults of both sexes and of known ages were measured in the following way. Heads were removed and fixed in the chosen fixative in a cavity slide and the length and breadth observed with a binocular microscope fitted with a calibrated scale in one eye piece. The length of each head was measured from the hindermost part of the epicranium to the labrum, and the breadth from a point between the compound eye and antennal socket on one side to a corresponding point on the other side. These measurements were of some assistance in the identification of the instar of nymphs from a large laboratory colony of P. americana, although in P. americana the number of instars (and hence the number of moults) varies and the increase in size of the head after each moult is so small that Dyar's law could not really be applied. Hence it is not possible to state with complete accuracy the instar of an individual taken at random.

Newly moulted specimens were normally selected, since it is easier to cut a non-tanned cuticle, but a few tanned nymphs belonging to third, fourth, and later instars were also used; the age in days of these

specimens since their last moult being recorded. First and second instar nymphs were killed at short intervals of time throughout each instar and a complete set of serial sections of their heads was prepared. Adults of both sexes of P. americana were selected at the following stages: newly emerged (within 1 hour after the moult and with their wings still crumpled), and 1 hour, 4 hours, 9 hours, 12 hours, 18 hours, 24 hours, 48 hours, 72 hours, 15 days, 126 days, and 127 days after the final moult. In a few cases, prior to fixation, the adult brain was dissected out of the head.

In the case of B. orientalis, nymphs were not reared individually but newly moulted nymphs of various sizes and adults of both sexes were taken from a large colony. Tanned B. orientalis nymphs and adults taken a certain time after moulting were also used. Heads of B. orientalis were measured in the same way as described for P. americana. Specimens were embedded in Steedman's (1947) ester wax by the following method.

1.	Specimens were brought to water or the appropriate alcohol depending on fixative, and washed for sufficient time to remove all fixative.	
2.	30% alcohol	4 hr
3.	50% alcohol	4 hr
4.	70% alcohol	overnight (15 - 16 hr)

5.	70% alcohol + cellosolve (equal parts)	2 hr
6.	Cellosolve	2 hr
7.	Cellosolve + ester wax (equal parts)	1 hr
8.	Ester wax (1)	1 hr
9.	Ester wax (2)	1-3 hr (depending on the size of the specimen)

Frontal and sagittal sections were cut at 6μ and stained by the chrome alum haematoxylin phloxin method of Gomori (1941) (Bargmann, 1949) and by the paraldehyde fuchsin stain of Gomori, 1950 (Gabe, 1953a) using Groat's haematoxylin and indigo carmine as counterstains. The material stained pink to purple-red by the latter method is hereafter referred to as PF+. Acid fuchsin and toluidine blue, acid fuchsin and trypan blue, and alcian blue and neutral red were also tried on a few serial sections of nymphal brain but proved to be of little value for this investigation. The two staining procedures used in the detection of neurosecretory material are given in detail below, the times being those which proved to be most suitable for the cockroach material. By far the best results have been obtained by the paraldehyde fuchsin staining procedure.

(1) The chrome alum haematoxylin phloxin stain of Gomori (1941) adopted by Bargmann (1949) as a neurosecretory stain

Preparation of the stain

2.5 gm. haematoxylin is dissolved in 250 ml. distilled water and to this is added a solution of 7.5 gm. chrome alum in 250 ml. distilled water. To this mixture are added 10 ml. of a 5% aqueous solution of potassium dichromate and 10 ml. of N/2 H_2SO_4. The mixture is ready for use after 48 hours.

Scott's tap water

As Glasgow tap water is relatively pure it is less effective as a rinse for blueing haematoxylin stained section, therefore, the sections had to be treated in alkaline Scott's tap water which is prepared by dissolving 3.5 gm. sodium bicarbonate and 20 gm. magnesium sulphate in 1,000 ml. distilled water.

Staining method

1. De-wax and bring sections gradually to water.
2. Refix in aqueous Bouin for 12-24 hours.
3. Wash in several changes of water to remove all traces of picric acid.

4. Oxidise sections in an aqueous solution of 0.3% potassium permanganate and 0.3% sulphuric acid for 1 minute.

5. De-colourize by rinsing very quickly (about 5 seconds) in 3% sodium bisulphite solution.

6. Rinse in distilled water.

7. Rinse in Scott's tap water.

8. Stain sections in chrome haematoxylin for 15 minutes.

9. Differentiate in acid alcohol for 25 seconds.

10. Treat sections in Scott's tap water in order to develop the blue colour of chrome haematoxylin.

11. Rinse in distilled water and counterstain with 0.5% aqueous phloxin stain. The sections now look red.

12. Rinse in distilled water for a short time and then put the slides in 5% aqueous phosphotungstic acid solution for about 2 minutes.

13. Rinse in distilled water and put in Scott's tap water until the sections again assume a bright red colour.

14. Rinse briefly in distilled water, followed by rapid dehydration in absolute alcohol.

15. Clear in xylol and mount in Canada balsam.

Results

Neurosecretory granules in the neurosecretory cells, in their axons, in the CC, and the CA, stain dark blue-black in colour. Some investigators have also identified

phloxinophilic material in these locations as neurosecretory. The ground cytoplasm stains purple, the nucleolus deep red, chromatin material black, muscle fibres pink, nerve fibres light blue, tracheae and tracheoles light blue to deep blue, neural lamella blue, and the covering sheath of the CC and the CA blue.

(2) The paraldehyde fuchsin stain of Gomori (1950) as adapted by Gabe (1953a) with counterstaining as recommended by Clark (Fraser, 1957)

Preparation of the stain

One millilitre conc. HCl and 1 ml. paraldehyde are added to 100 ml. of 0.5% aqueous solution of basic fuchsin and the solution is kept in a stoppered bottle at room temperature for at least 4–5 days. Each day a 'spot test' is applied on a piece of filter paper. It can be seen that at first too much basic fuchsin appears in the centre of the blot, but this diminishes gradually as the solution ripens. In a fully mature solution there is no longer a trace of basic fuchsin in the centre of the blot. The solution is now ready for use. It is filtered and the residue on the filter paper is kept, while the filtrate is discarded. The residue is dissolved in 100 ml. of 70% alcohol. This is the stock solution which can be stored at 4°C for as long as a

month. For staining, 25 ml. is taken from the stock solution, and 75 ml. of 70% alcohol and 1 ml. of glacial acetic acid are added. This mixture is freshly prepared on the day of use.

Preparation of Groat's haematoxylin

0.8 ml. conc. H_2SO_4 is mixed with 1 gm. iron alum in 50 ml. distilled water. 50 ml. 90% alcohol and 0.5 gm. haematoxylin are added to this mixture, and the whole is shaken well until the solids are dissolved. The mixture is filtered, the fine precipitate rejected and the filtrate is stored in a glass stoppered bottle and is ready for use the next day.

Staining method

1. De-wax and bring the sections gradually to water.

2. Oxidise sections for 1 minute in an aqueous solution containing 0.3% sulphuric acid and 0.3 potassium permanganate in equal parts. This oxidising solution should be discarded after one hour and a new solution prepared.

3. Rinse in water and put the slides in 2.5% aqueous sodium bisulphite solution till the sections become white. It usually takes 40-45 seconds to decolourise them. Care should be taken not to keep the sections any longer in this reducing solution after they become white otherwise the reaction will be reversed and the neurosecretory material will not stain with paraldehyde fuchsin. On the other hand, if the

sections are not allowed to become completely white, the presence of the slightest tinge of yellow colour will interfere with the staining. This is the critical stage in the whole staining procedure, where great caution is needed.

4. Wash in running tap water for about 3 minutes.

5. Stain in paraldehyde fuchsin solution for about 5 minutes.

6. Differentiate in acid alcohol for about 5 minutes.

7. Rinse briefly in Scott's tap water.

8. Stain sections in Groat's haematoxylin for about 2 minutes.

9. Wash in fresh Scott's tap water for nearly 30 seconds.

10. Rinse briefly in distilled water.

11. Stain for about 30 seconds in a solution of 0.25 gm. indigo carmine in 100 ml. saturated aqueous solution of picric acid.

12. Drain off the excess indigo carmine solution. Blot dry. Dehydrate the sections rapidly in absolute alcohol where they will lose their excess green colour.

13. Clear in xylol and mount in Canada balsam.

Results

Nuclear and cell membranes, the cytoplasm of the ordinary neurons and the neuropile stain grey. The

chromatin material stains black, sometimes grey or green. The general background stains grey or green. The muscle fibres are green. Neuorsecretory material stains a brilliant purple colour. The cytoplasm of certain neurosecretory cells may, at some stages in the secretory cycle, appear to stain a uniform faint pink or purple colour but generally the cytoplasm stains grey. The coarse granules in the ubiquitous haemocytes, the large gliosomes of glia cells, part of the cuticle of tracheae and tracheoles, newly laid down cuticle, intima of the gut, and the neural lamella stain pink to purple-red. Other staining procedures used for the study of the morphology of the nervous system include haemalum and eosin staining, ironalum and Heidenhain's haematoxylin, the modified haematoxylin method of Wigglesworth (1953), Bodian's activated protargol technique suggested by Powers (1943), Romanes (1950) silver method, and the osmium ethyl galate method of Wigglesworth (1957b, 1959b).

OBSERVATIONS

Section I: Neurosecretory Cells in the Protocerebrum

In each half of the protocerebrum of the 42-day embryo of P. americana there are not fewer than nine

Figure 53. Diagram of the protocerebrum of a 42-day embryo of P. americana showing the location of median, lateral and ventral neurosecretory cells. MNSC, median neurosecretory cell; LNSC, lateral neurosecretory cell; Ext.C, external calyx of corpora pedunculata; Int.C, internal calyx of corpora pedunculata.

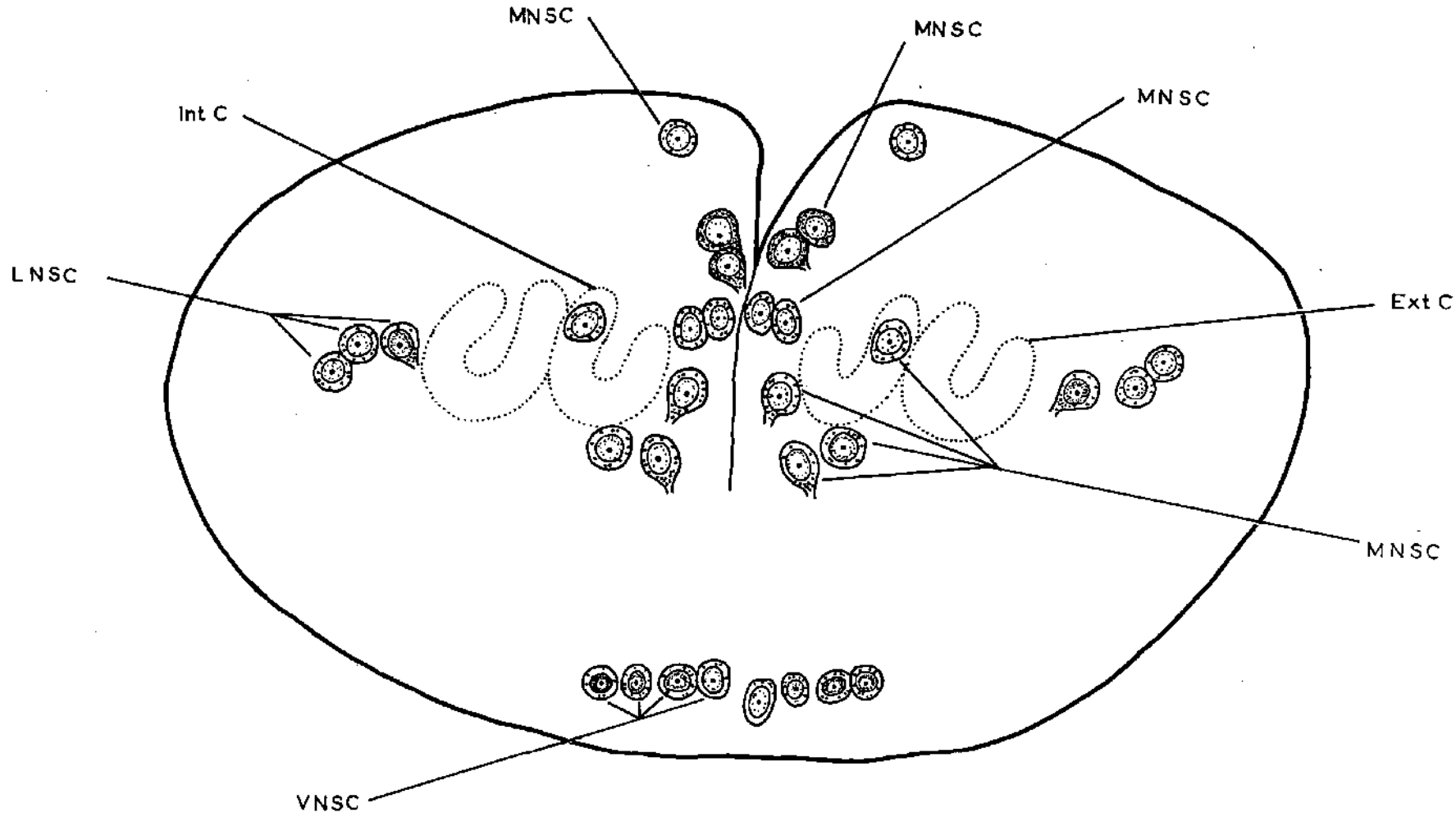


Figure 53.

active NSC in the median pars intercerebralis, three in the lateral part of the protocerebrum, and four ventral to the protocerebral neuropile (Fig. 53). These can be termed median, lateral and ventral (or paraconnectivus, see Chapter 4) NSC, respectively. It may be noted here, although it will be discussed more fully later, that any neuron which is seen to contain secretory granules may be regarded as an active neurosecretory cell.

In addition to a diffuse purple-red colouration of the cytoplasm, PF^+ granules are evident in the perikaryon and in the axon-hillock of these cells. In the 50-day embryo none of these neurons possess PF^+ material although several can readily be identified. Thus a neuron with large vacuoles located ventro-lateral to the internal calyx of the corpus pedunculatum in the median pars intercerebralis can still be identified by its typical shape, size and position (Fig. 23). This cell in the 42-day embryo would have had purple-red cytoplasm and sparse purple-red granules in the perikaryon and axon-hillock (Fig. 53). In the 50-day embryo there is also one lateral NSC with sparse PF^+ granules in its cytoplasm. In the 54-day embryo five median NSC and one lateral NSC, containing PF^+ granules, occur in each half of the protocerebrum (Fig. 54).

Figure 54. Diagram of the protocerebrum of a 54-day embryo of P. americana showing the median and lateral neurosecretory cells. Lettering as in Figure 53.

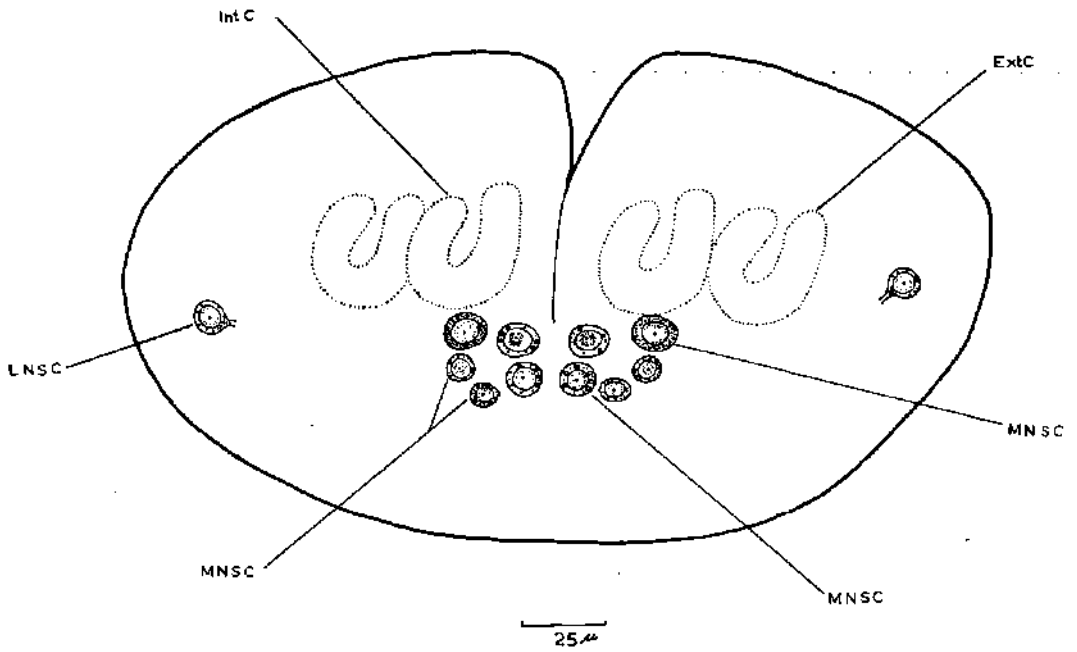


Figure 54.

In the newly hatched (half-hour) first instar nymph nine median NSC with PF^+ granules and small vacuoles in the cytoplasm occur in each half of the brain (Fig. 55). The activity of these particular cells in freshly hatched nymphs must, of course, have commenced in late embryonic life but active median NSC in various loci can be seen throughout the 12½ days' duration of the first instar. PF^+ granules occur commonly in the axons of the NCCI, this material becoming particularly abundant in the latter days (8-12) of the first instar, indicating an increase in the flow of neurosecretory material in the premoult period. Certain of these cells which have a precise location are known to have phases of activity in the late embryo and again in the first instar. For example, two median NSC located near the anterior limit of the protocerebrum and active in the 42-day embryo (Fig. 53), appear again in the first instar, four hours after hatching; while a large cell ventral to the internal calyx of the corpus pedunculatum, which contains abundant PF^+ material in the 54-day embryo (Fig. 54), appears again in the eighth day of the first instar. The lateral NSC could not be seen in the first instar brain until the third day, persisting until the eighth day, but PF^+ granules are found in the NCCII for some days after this. Lateral NSC have now increased in number

Figure 55. Diagram of the protocerebrum of a first instar nymph (½ hr old) of P. americana, showing median neurosecretory cells. Lettering as in Figure 53.

Figure 56. Diagram of the protocerebrum of a first instar nymph (5 days old) of P. americana, showing median, lateral and ventral neurosecretory cells. Letting as in Figure 53.

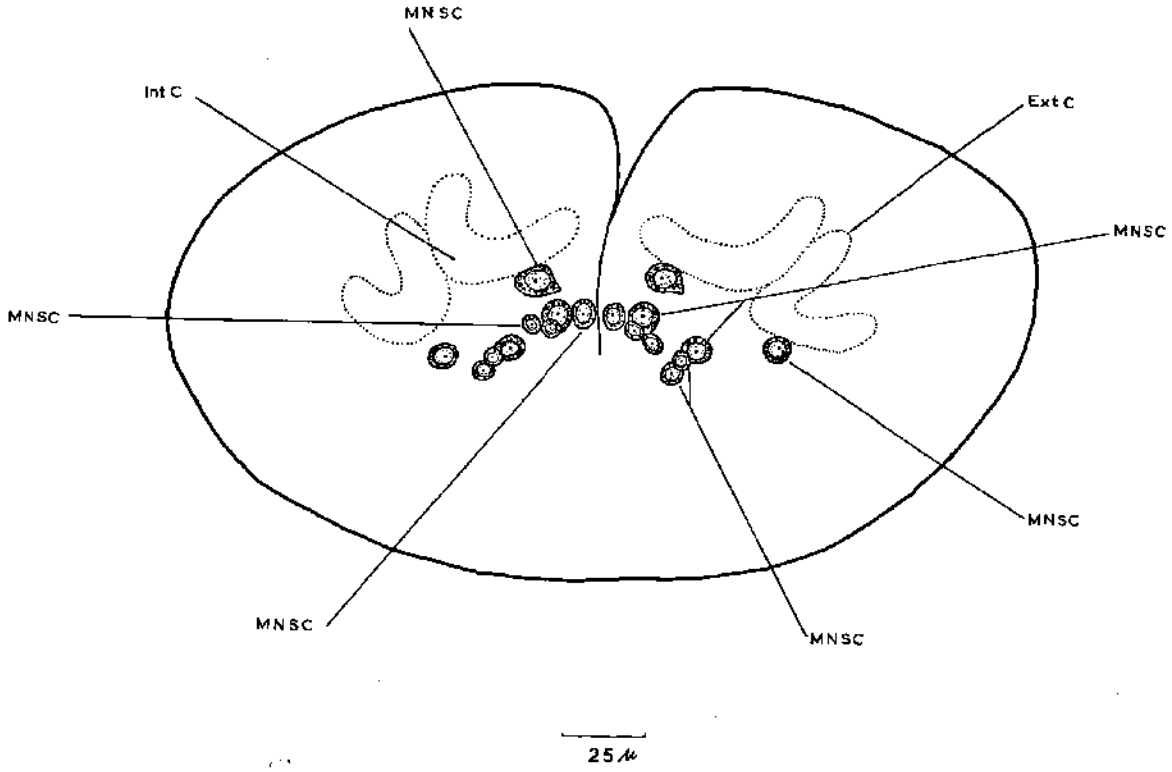


Figure 55.

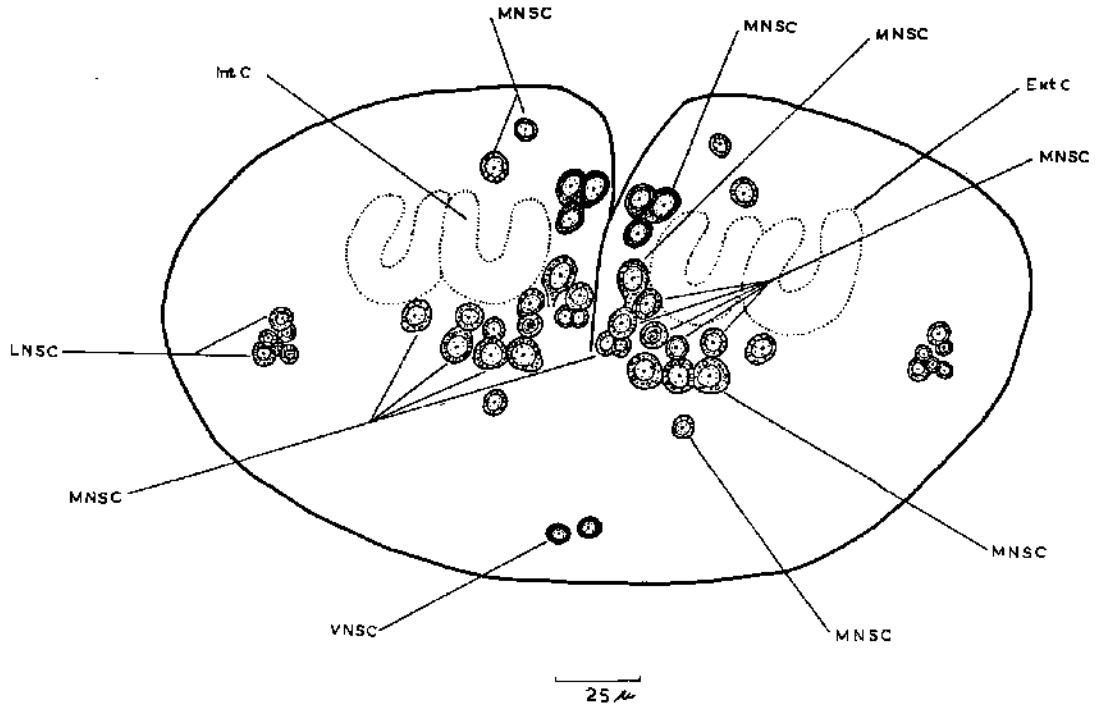


Figure 56.

to six or eight in each half of the brain (Fig. 56). Ventral NSC which were noted for the first time in the 42-day embryo appear again in 5-8 day-old instar nymphs (Fig. 56).

Median, lateral and ventral NSC can also be found in the protocerebrum of later nymphal instars in which they show similar definite cycles of activity. They also occur in adults of both sexes. Medium-sized and small NSC, some of which are full of PF^+ granules, can be seen in the brains of newly moulted nymphs (Fig. 57) and newly moulted adults of both sexes. These cells are obviously homologous with the median NSC already noted in the first instar. An attempt to classify the NSC of the protocerebrum further is made in Chapter 4 of this work.

In newly moulted adult females some of these median cells lose all their secretory granules about 18 hours after the moult and although median NSC containing sparse PF^+ secretory granules can be found in young adult brains, PF^+ material does not again become abundant in these cells until sexual maturity is attained. Brains of individuals of both sexes taken in copulation contain numerous prominently stained NSC.

The numbers of median, lateral and ventral NSC

Figure 57. Photomicrograph of frontal section of the brain of a last instar ♀ nymph of P. americana, 1 hour after moult, showing median neurosecretory cells (MNSC), lateral globuli cells (LGC) of corpora pedunculata, a portion of the internal calyx (Int.C) of corpora pedunculata. Note the presence of PF^{+} granules all along the axon of a median neurosecretory cell.

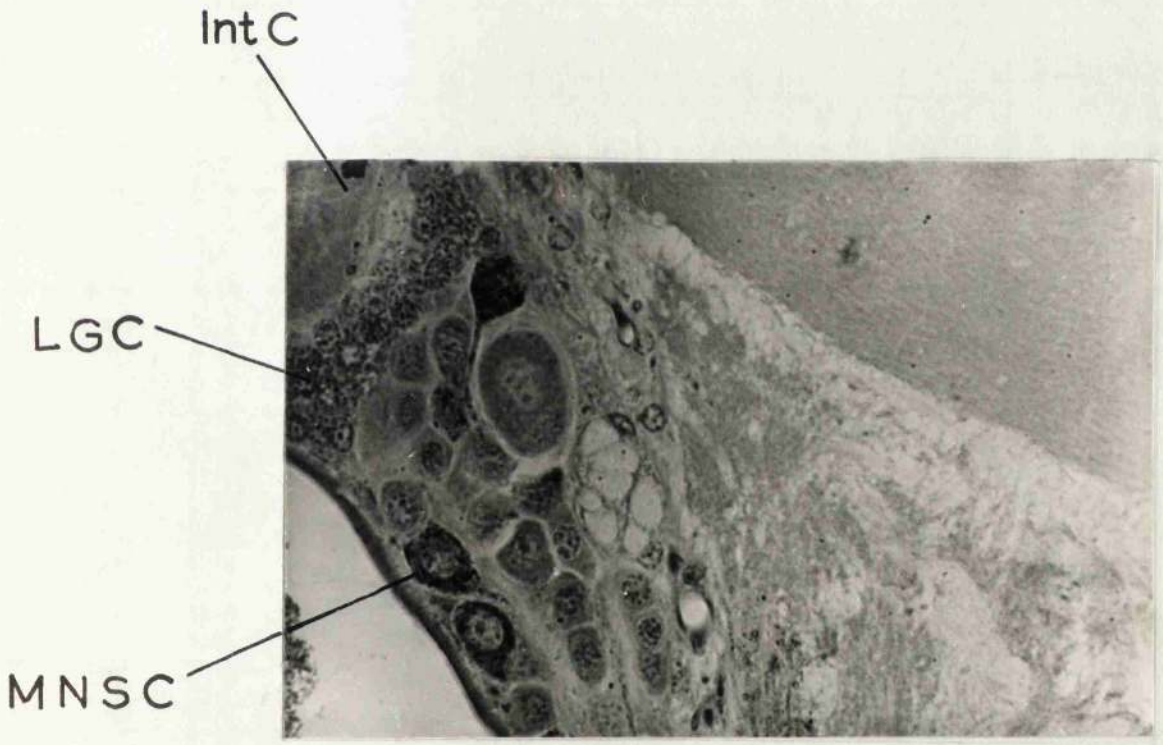


Figure 57.

vary from individual to individual in a given nymphal instar and in the adult stage. Füller (1960) asserts that there are 18-20 NSC in the protocerebra of second instar nymphs, 30 NSC in third instar nymphs, and 45-50 NSC in last instar nymphs and adults of P. americana. His estimate of the number of NSC in second instar is certainly low and for NSC in adults it is very low. The number of NSC found in the brains of individuals of the same age and apparently in the same physiological state can vary, but more marked and much more significant, is the variation in the number of NSC found at different stages in a given instar. NSC have been observed and counted in all stages from the embryo to the adult. The most complete record has been obtained for the NSC of first instar nymphs (see Table 1). From this it can be seen that the maximum number of NSC observable in the protocerebrum in the first instar increases from seven, shortly after the moult, to a maximum of twenty-five on the fifth day, and declines sharply after the eighth day. A similar increase and decrease in number can be observed in the later instars. The total number of active NSC is greater in each successive instar so that, for example, it is possible to find in each half of the protocerebrum of a last instar female nymph, which has

just moulted, no fewer than 82 median NSC alone; while in the adult the number of median NSC may reach 180 just after the moult. At the same time the number of lateral NSC will be about 30 and of ventral NSC 10. Table 2 indicates the numbers of NSC observable in the protocere-:bra of females at different stages of adult life. The difference between the last two adults of approximately the same age can be related to differences in reproductive activity. Some of the smaller and medium-sized median NSC appear to be packed with neurosecretory material. This is never so in the case of lateral and ventral NSC. Thus in the median NSC there appear to be two phases of activity, firstly, a storage phase in which the rate of accumulation is much greater than the rate of discharge and, secondly, a discharge phase when the accumulation or synthesis of neurosecretory material has ceased. On the other hand, it seems reasonable to assume that when the lateral and ventral NSC are active they produce neurosecretory material which is rapidly discharged without being stored in the perikaryon. Contrary to a recent statement by Highnam (1961) concerning NSC in Schistocerca, vacuoles of different sizes have been observed in some of the NSC of P. americana, at certain stages of development after paraldehyde fuchsin staining.

Table 1. NSC in the protocerebrum of the first instar nymphs.

Age	No. of median NSC in one half of protocerebrum	No. of lateral NSC in one half of protocerebrum	No. of ventral NSC in one half of protocerebrum	Total no. of NSC of protocerebrum in one half of the brain
½ hr	9	-	-	9
4 hr	7	-	-	7
1 day (24 hr)	8	-	-	8
3 days	11	7	-	18
5 days	18	6	1	25
8 days	11	8	2	21
9 days	4	-	-	4
10½ days	2	-	-	2
12½ days	2	-	-	2

- not recorded

Table 2. NSC in the protocerebrum of adult females

Age	No. of median NSC in one half of the protocerebrum	No. of lateral NSC in one half of the protocerebrum	No. of ventral NSC in one half of the protocerebrum	Total no. of NSC in one half of the protocerebrum
Adult female killed within 1 hr after last moult	180	30	10	220
Adult female killed 1 hr after last moult	160	30	10	200
Adult female killed 12 hr after last moult	156	20	8	184
Adult female killed 18 hr after last moult	141	25	10	175
Adult female killed 48 hr after last moult	132	9	6	147
Adult female killed 72 hr after last moult	114	8	10	132
Adult female killed 15 days after last moult	84	-	-	84
Adult female mating (age?)	184	-	-	104
Adult female killed 126 days after last moult	9	-	6	15
Adult female killed 127 days after last moult	122	-	-	122

- not recorded

Figure 58. Photomicrograph of the frontal section of the brain of a last instar ♀ nymph of P. americana, killed 1 hour after moulting, showing abundant PF^+ granules in two roots (R1, R2) of the NCCI. PRCNp, protocerebral neuropile; MNSC, median neurosecretory cell; LGC, lateral globuli cells; Int.CX, a portion of the internal calyx.

Figure 59. Photomicrograph of the frontal section of the brain of an adult ♀ P. americana, killed 18 hours after moulting, showing the NCCI and its two roots. Lettering as in Figure 58.

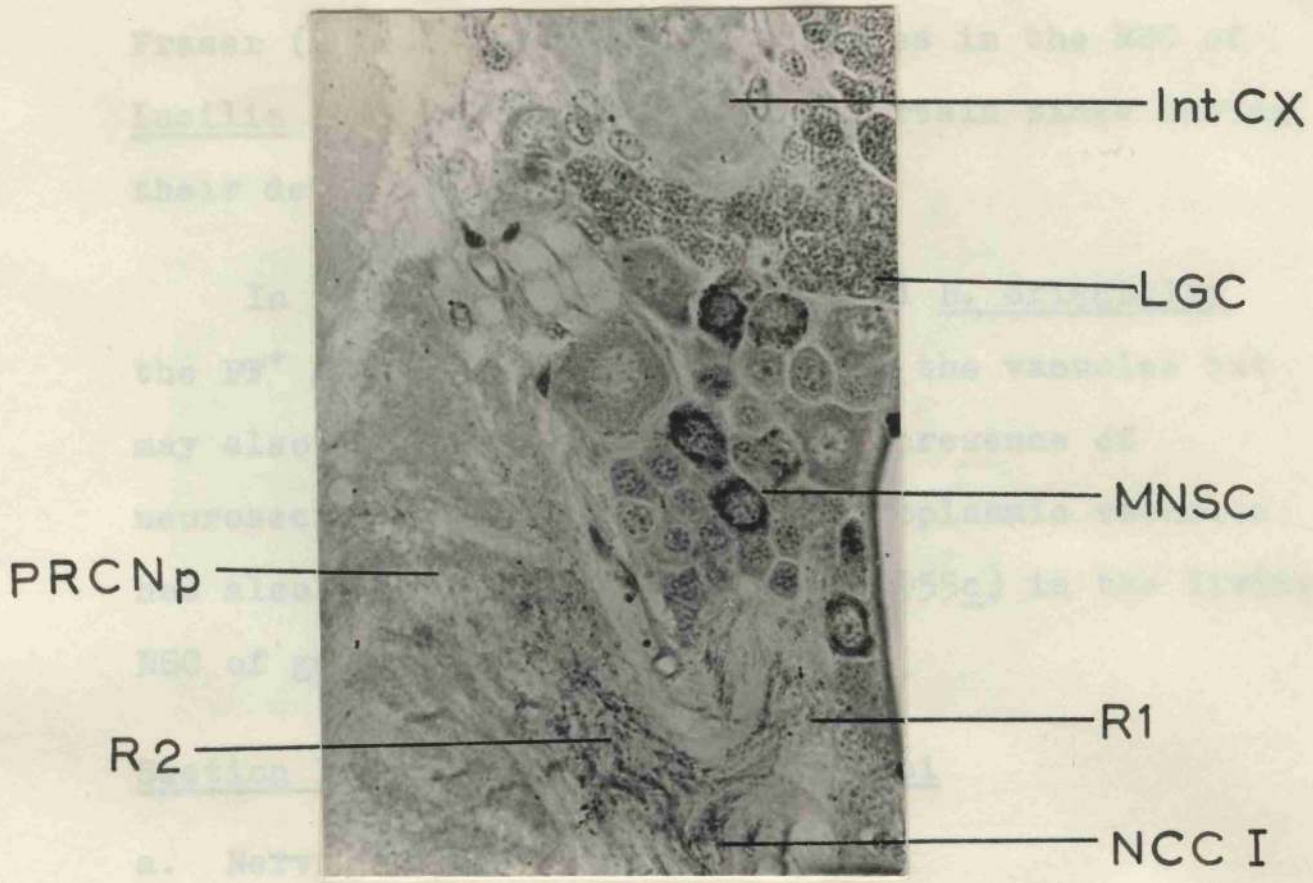


Figure 58.

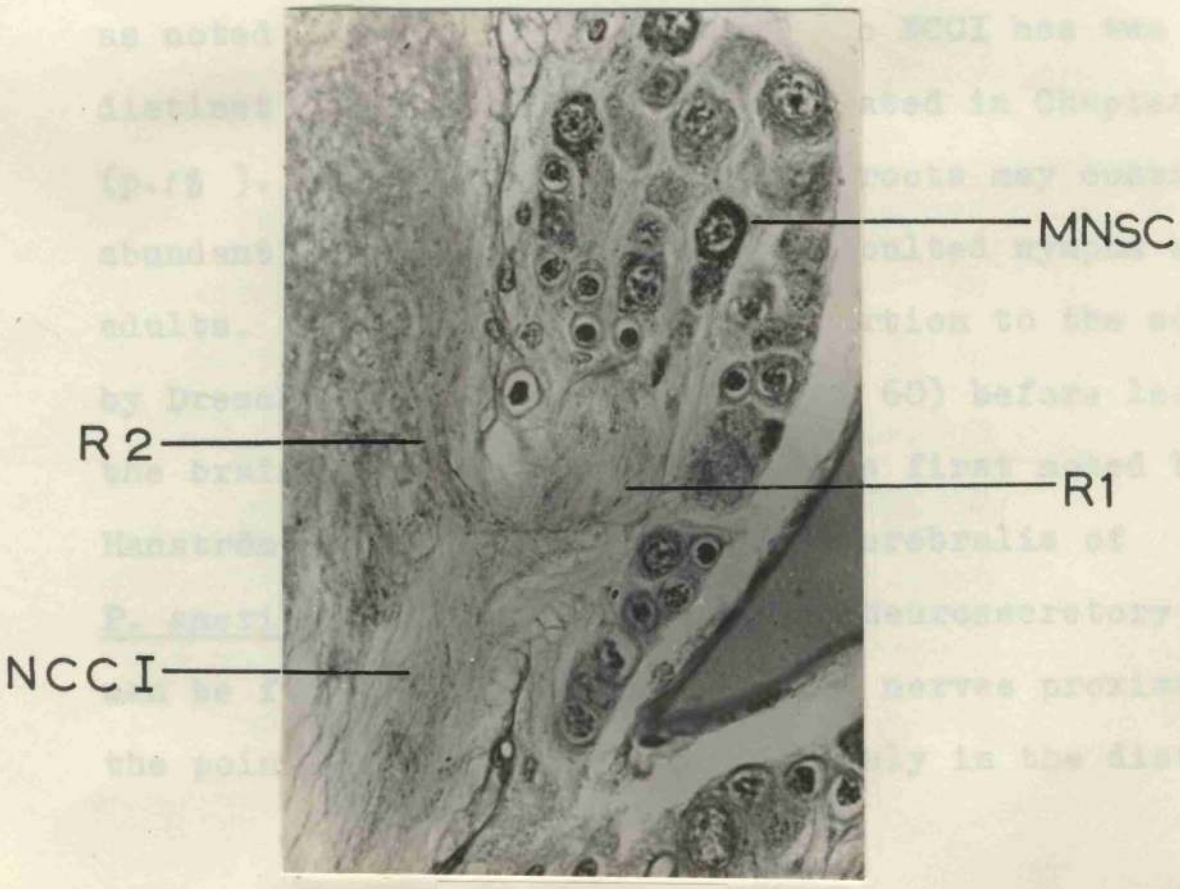


Figure 59.

Fraser (1957) also observed vacuoles in the NSC of Lucilia and Calliphora larva at certain times during their development.

In the NSC of P. americana and B. orientalis the PF^+ granules not only surround the vacuoles but may also occur inside them. The presence of neurosecretory granules in the cytoplasmic vacuoles has also been reported by Nayar (1955c) in the living NSC of gall midges.

Section II: Nervi Corporis Cardiaci

a. Nervi corporis cardiaci interni

The NCCI is formed by the axons of the median NSC as noted above. The prechiasmatic NCCI has two distinct roots (Figs 58, 59) as stated in Chapter 1 (p./8). Axons in each of these roots may contain abundant PF^+ granules in freshly moulted nymphs and adults. The NCCI despite an assertion to the contrary by Drescher (1960), do cross (Fig. 60) before leaving the brain from its ventral side, as first noted by Hanström (1940) in the pars intercerebralis of P. americana and B. orientalis. Neurosecretory material can be found in the axons of these nerves proximal to the point of crossing, and not solely in the distal

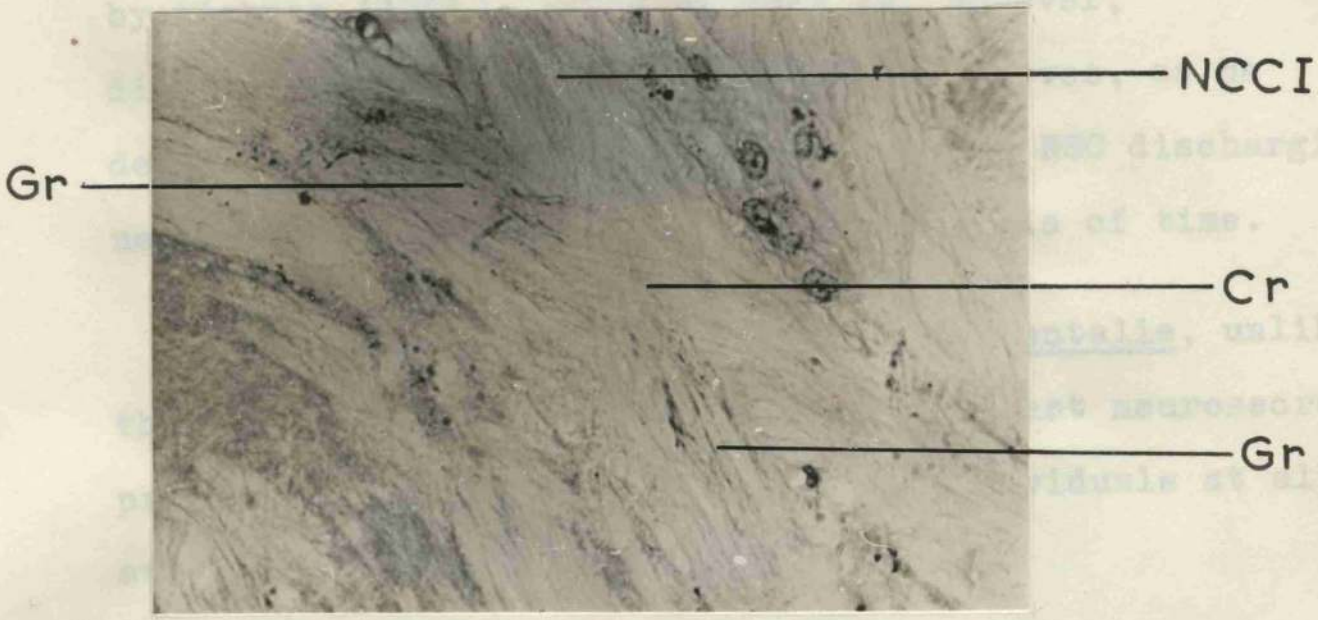


Figure 60.

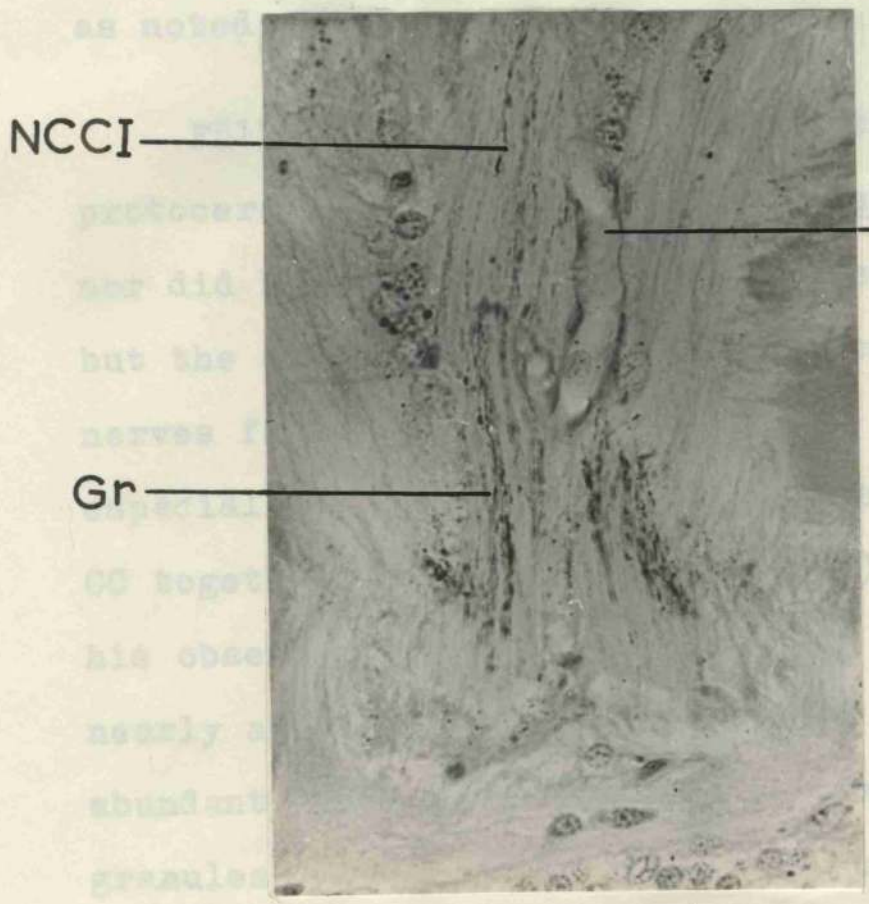


Figure 61.

parts of the nerves as recently recorded in Schistocerca, by Highnam (1961). The product is, however, discontinuously distributed along the nerves, as he described, indicating phasic activity of NSC discharging neurosecretory material at short intervals of time.

The NCCI of P. americana and B. orientalis, unlike those of Schistocerca, may contain abundant neurosecretory products (Fig. 61) in newly moulted individuals at all stages including immature adults.

b. Nervi corporis cardiaci externi

The NCCII is formed by the axons of lateral NSC as noted above.

Füller (1960) did not find lateral NSC in the protocerebrum of either P. americana or B. orientalis nor did he find neurosecretory material in the NCCII; but the author has recorded its occurrence in these nerves for the first time (in both species of cockroach) especially when they enter the anterior part of the CC together with the NCCI (Fig. 62), in accordance with his observations on lateral NSC in late embryos and in nearly all instars. The NCCI and NCCII may have abundant peripheral and some central neurosecretory granules when these nerves are entering the CC.

Figure 62. Photomicrograph showing neurosecretory granules (Gr) in the extra-cerebral nervi corporis cardiaci interni (NCCI) and nervi corporis cardiaci externi (NCCII) of an adult ♀ P. americana killed 4 hours after the moult. Note also the anterior part of the corpus cardiacum (CC).

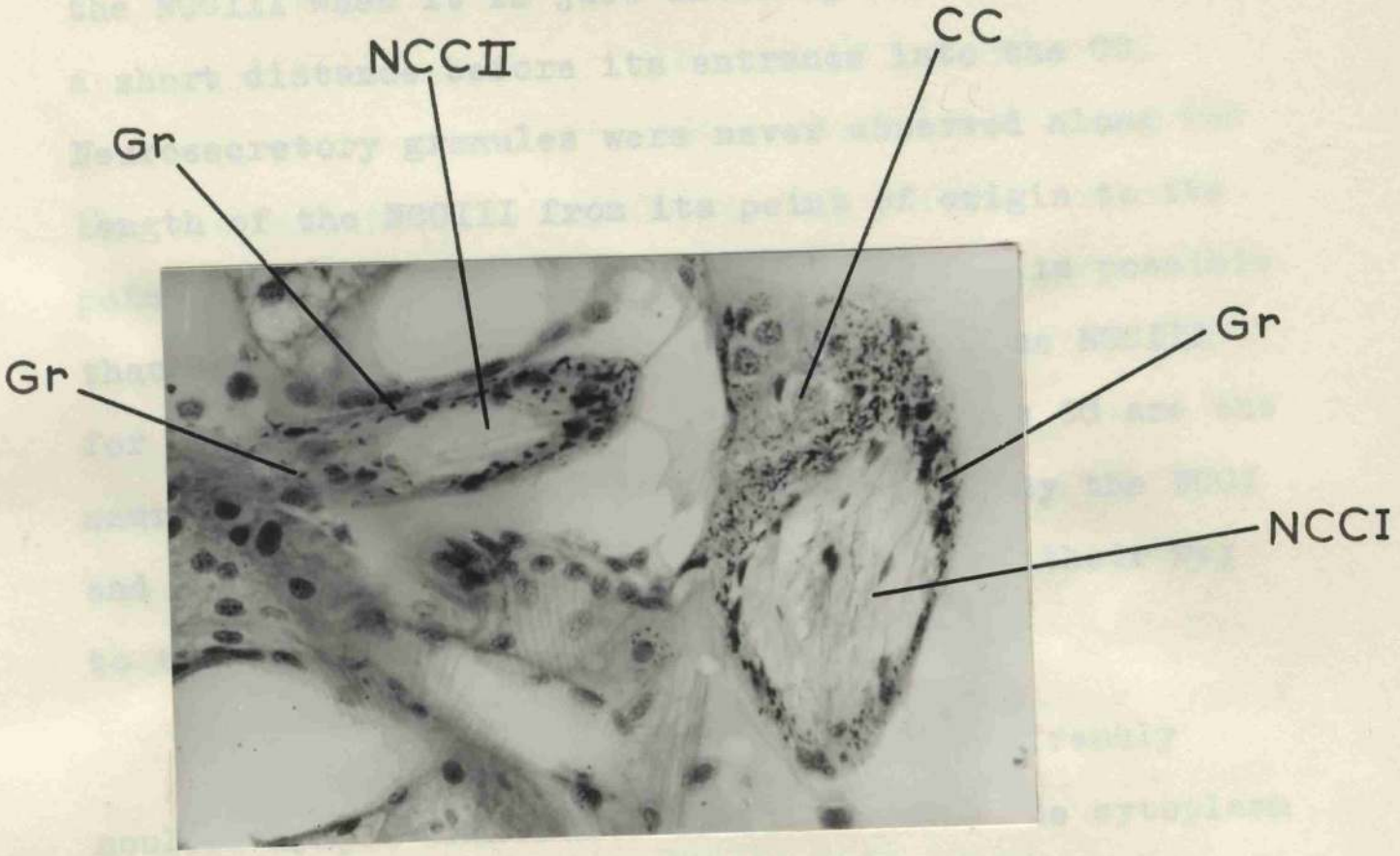


Figure 62.

c. NCCIII

NCCIII arises from the inner antero-lateral part of the tritocerebrum and innervates the CC (see Chapter 1). Neurosecretory material may be seen in the NCCIII when it is just entering the CC or even for a short distance before its entrance into the CC. Neurosecretory granules were never observed along the length of the NCCIII from its point of origin to its point of entry into the CC. Therefore it is possible that the neurosecretory granules seen in the NCCIII for a short distance in the vicinity of the CC are the neurosecretory granules of the CC brought by the NCCI and NCCII, and that these granules were on their way to the tritocerebrum via the NCCIII.

Though in the 42-day embryo and some freshly moulted nymphs and adults of both sexes, the cytoplasm of a few cortical nerve cells of tritocerebrum stains diffuse light purple and contains a few PF^+ granules (see Chapter 4), and in spite of the fact that sometimes in overstained sections some of the axons of the tritocerebrum and a few axons of the NCCIII stain diffuse purple, the author thinks that at present it would be unwise to assign to these nerve cells a definite neurosecretory function because no neurosecretory cycle was observed in them (see Chapter 4); it would

also be jumping to an as yet unwarranted conclusion to state here that neurosecretory material is transported from tritocerebrum via the NCCIII to the CC, because no distinct PF^+ or CH^+ granules have been observed throughout this nerve. However, if in cockroaches there is a transport of neurosecretory material from the tritocerebrum to the CC via the NCCIII, as shown by Dupont-Raabe (1956, 1957, 1958) in Carausius, the amount of neurosecretory material might be very small and perhaps transported in a diffuse form. This is merely a possibility. However, the problem of neurosecretion in the tritocerebrum in cockroaches certainly needs further study (see Chapter 4). The problem of transport of neurosecretory material either to or from the tritocerebrum (both possibilities should be kept in mind) via the NCCIII also needs further investigation, especially by ligaturing this nerve to observe histologically whether the maximum accumulation of neurosecretory material is in that part of the NCCIII which is towards the tritocerebrum and can receive neurosecretory material from it alone or is on the other side, which can obtain neurosecretory material from the CC alone. In this connection, perhaps it would not be out of place to mention that Willey (1961) could not find neurosecretory material

in the NCCIII of cockroaches he investigated, either.

Section III: Corpora Cardiaca

In P. americana and B. orientalis the CC are paired structures. They are oviform, or pear-shaped, and lie behind the brain, above the gut, close to the aorta and on either side of the recurrent nerve (see Chapter 1 above).

During the earlier part of the embryonic development of P. americana the development of the CC is gradual and continuous, but in the 42-day embryo they are notably enlarged and contain, for the first time, fine PF^+ granules (Figs 50, 51). There is a subsequent decline in size in 50 and 54-day embryos accompanied by a marked decrease in the amount of neurosecretory material stored in these organs.

The CC of a half-hour-old first instar nymph (Fig. 63) are smaller than those of a 42-day embryo and contain a few minute PF^+ granules; but within four hours after hatching they start to increase in size and the PF^+ granules become coarse and dense, and increase in size and number. Thereafter the CC gradually increase in volume until maximum size is attained at the sixth day and maintained until the eighth day (Fig. 64). The volume then declines towards

Figure 63. Photomicrograph of a frontal section of the corpora cardiaca (CC) of a half-hour-old first instar nymph of P. americana showing a few PF-positive neurosecretory granules (Gr). RN, recurrent nerve; Ao, aorta; Oes, oesophagus.

Figure 64. Photomicrograph of a frontal section of the corpora cardiaca of an 8-day-old first instar nymph of P. americana showing PF-positive neurosecretory material (Gr) in the nerve endings. The corpora cardiaca have increased in size. Lettering as in Figure 63.

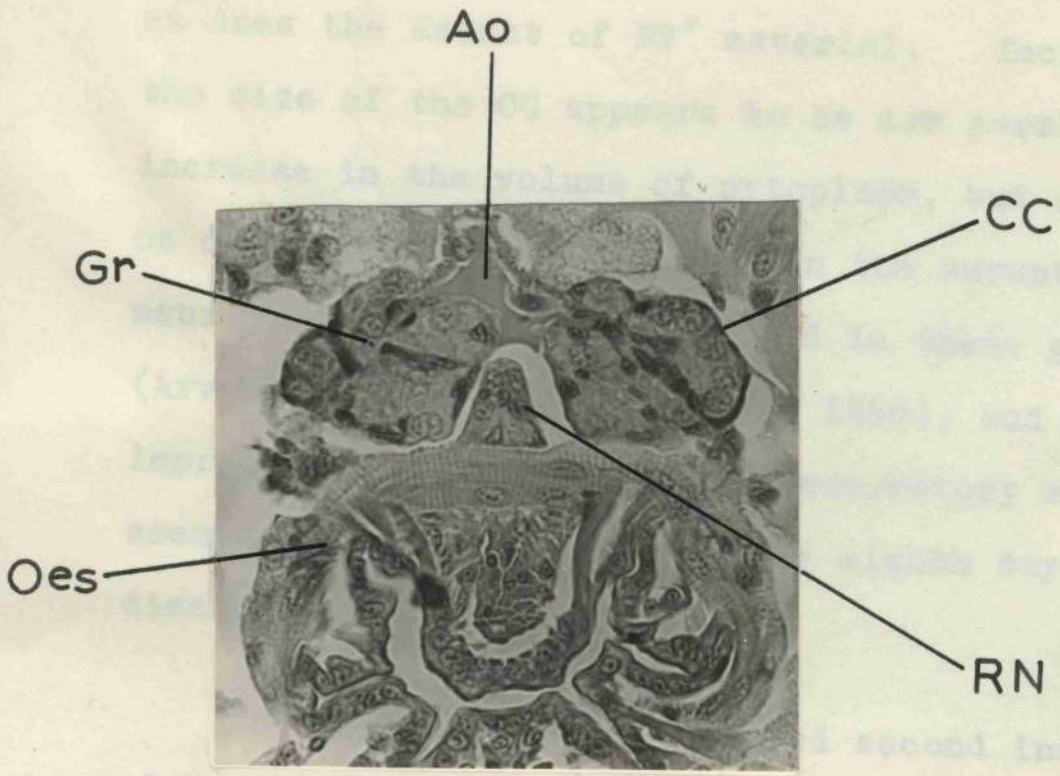


Figure 63.

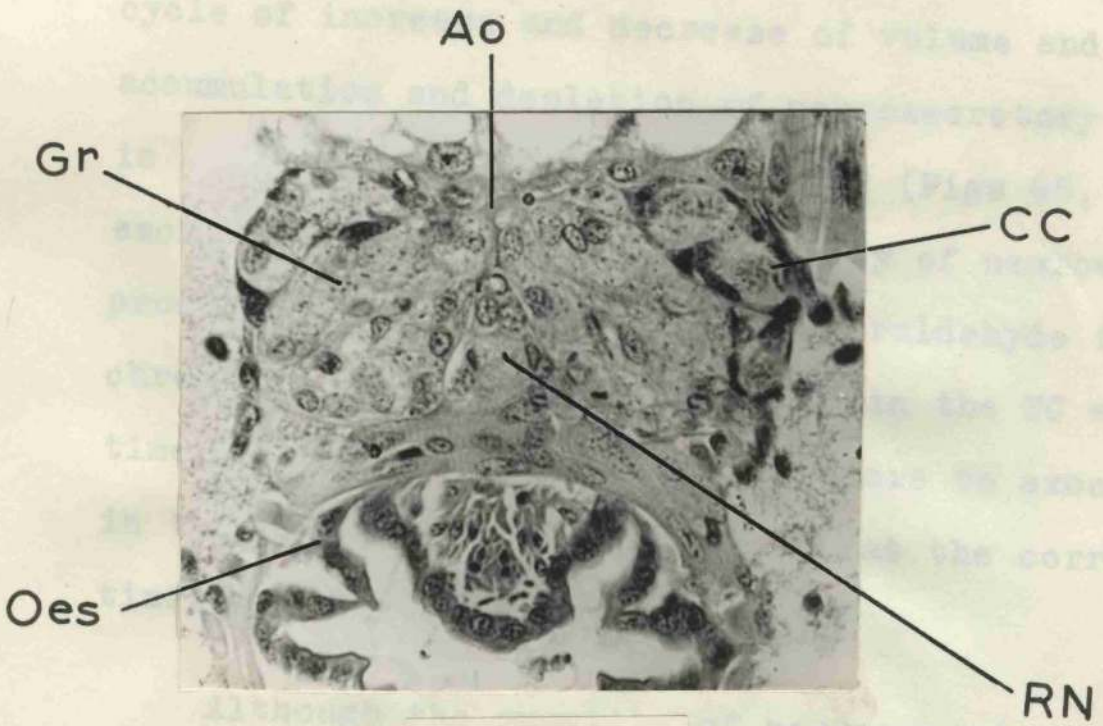


Figure 64.

the end of the instar, i.e. about the thirteenth day, as does the amount of PF^+ material. Increase in the size of the CC appears to be due partly to an increase in the volume of cytoplasm, but could also be due partly to an increase in the amount of neurosecretory substance stored in these glands (Arvy & Gabe, 1952b; Highnam, 1958), and the impression is gained that neurosecretory material accumulates in the CC up to the eighth day and is then discharged.

Again, in the newly moulted second instar nymphs of P. americana, the CC are at first smaller than they were at their peak in the previous instar. The same cycle of increase and decrease of volume and of accumulation and depletion of neurosecretory material is repeated in all nymphal instars (Figs 65, 66). In each successive instar the quantity of neurosecretory product, demonstrable either by paraldehyde fuchsin or chrome haematoxylin staining, within the CC at the time of maximum accumulation, appears to exceed that in the CC of the preceding instar at the corresponding time.

Although the quantity of neurosecretory material

Figure 65. Photomicrograph of the frontal section of the corpora cardiaca of a third instar nymph of P. americana about the stage of maximum accumulation of neurosecretory material. Paraldehyde fuchsin stain. CC, corpora cardiaca; Ao, aorta; RN, recurrent nerve; Gr, PF-positive neurosecretory material.

Figure 66. Photomicrograph of the frontal section of the corpus cardiacum of a freshly moulted nymph of B. orientalis. Chrome haematoxylin phloxin stain. CC, corpus cardiacum; DC, dorsal commissure; Gr, CH-positive neurosecretory material.

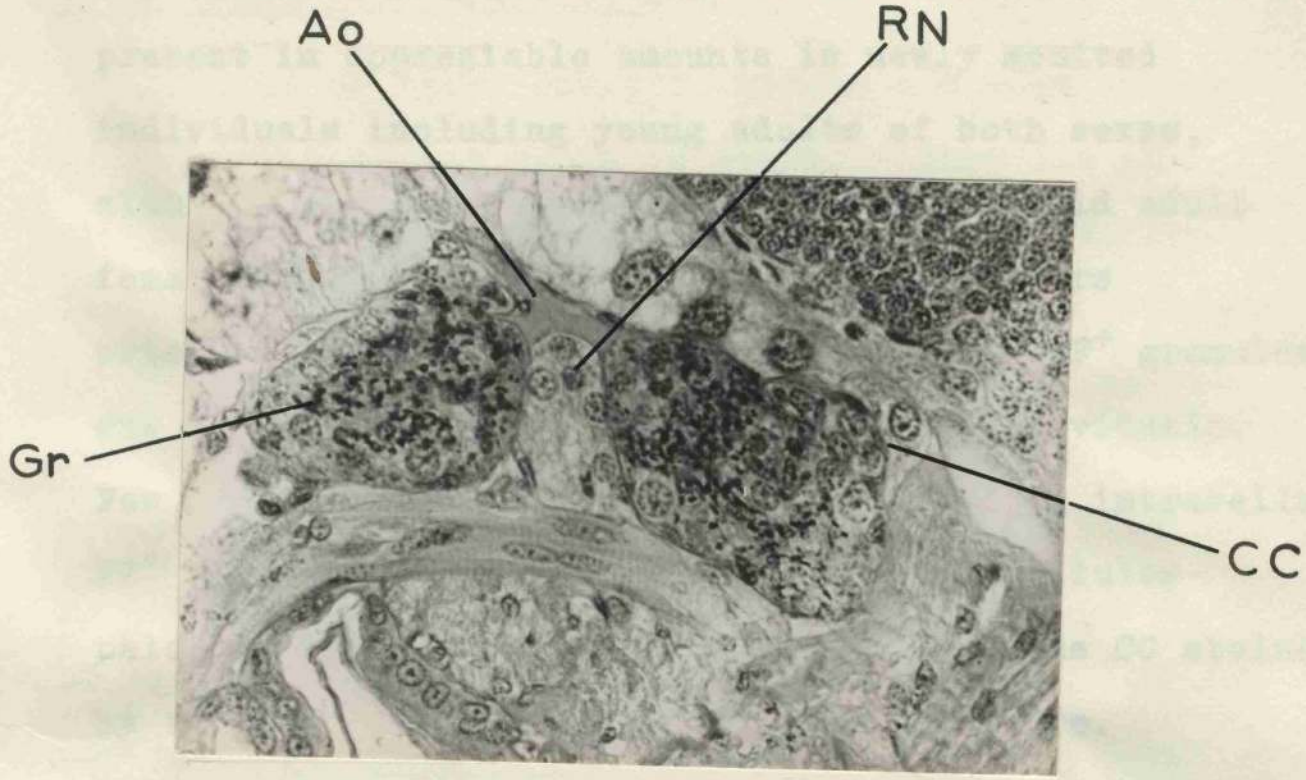


Figure 65.

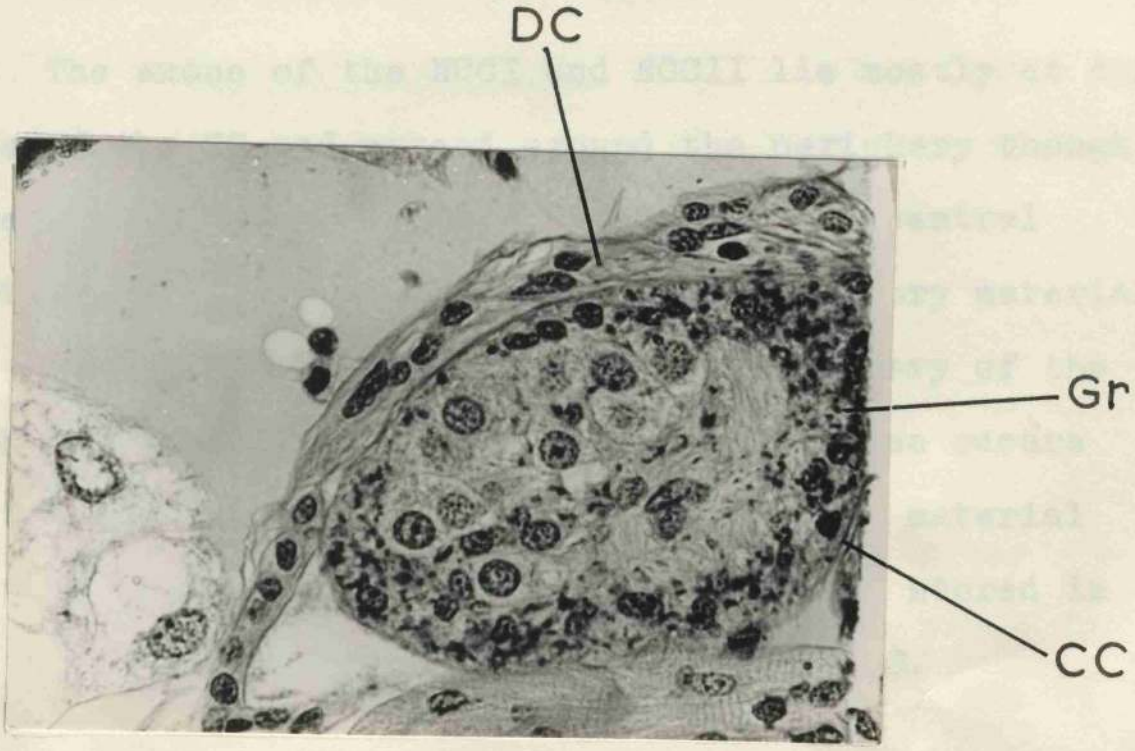


Figure 66.

may diminish towards the end of an instar it is still present in appreciable amounts in newly moulted individuals including young adults of both sexes, although there is less in, say, a 72-hour-old adult female than in one which had moulted 48-hours prior to fixation of its head. Finally, PF^+ granules are again abundant in the CC of mating individuals. Few large cells of the CC appear to contain intracellular PF^+ granules. Neither inter- nor intracellular phloxinophilic material could be seen in the CC stained by the chrome haematoxylin phloxin procedure. Phloxinophilic intrinsic secretion of the CC has been described by Stukinsky (1952) and Füller (1960) in cockroaches, Ozeki (1958b) in Anisolabis and Highnam (1961) in Schistocerca.

The axons of the NCCI and NCCII lie mostly at the base of the CC and extend around the periphery though, some of the axons are also present in the central part of the glands. Thus the neurosecretory material is usually found mostly towards the periphery of the CC but when present in large amounts it also occurs in the central part. This neurosecretory material is to be found between the cells of the CC stored in the swollen termini of the axons of the NCC.

Section IV: Corpora allata

The CA of later embryos, nymphs and most adults of P. americana are ovoid in shape. They lie on either side of the hypocerebral ganglion and dorso-lateral to the gut. Owing to a considerable increase in size, they tend to shift downwards lateral to the gut in the sexually mature adult female. In the locust embryo the oval CA are said, by Jones (1956), to assume after katatrepsis their 'customary position' on the 'lateral side' of the gut. In the 42-day embryo of P. americana the CA are not in this position which is occupied by deceptively similar muscle block and there are, as might be expected (Wigglesworth, 1956), several haemocytes containing large PF^+ granules associated with the developing muscles. The location of the CA in Locusta migratoria give by Ozbas (1957) agrees with the author's location of these organs in P. americana.

Füller (1960) did not find neurosecretory granules in the CA of P. americana either in the embryos or in 1st, 2nd or 3rd instar nymphs but in the present observations PF^+ granules can first be seen in the CA on the forty-second day of embryogenesis, becoming commoner in 50 and 54-day embryos (Fig. 52). The CA

Figure 67. Photomicrograph of the frontal section of the corpora allata (CA) of a 7-day-old 1st instar nymph of P. americana, showing a few PF^+ neurosecretory granules (Gr). Hg, hypocerebral ganglion; Ao, aorta; Oes, oesophagus.

Figure 68. Photomicrograph of the frontal section of the corpora allata of a 7-day-old 1st instar nymph immediately posterior to the section shown in Figure 67. Note the presence of PF^+ granules in the corpora allata. Lettering as in Figure 67.

Figure 69. Photomicrograph of the frontal section of the corpora allata of a 12-day-old 1st instar nymph of P. americana showing abundant PF^+ neurosecretory granules. Lettering as in Figure 67.

Figure 70. Photomicrograph of the frontal section of the corpora allata of a 7-day-old 2nd instar nymph of P. americana showing abundant PF^+ neurosecreoty granules. Lettering as in Figure 67.

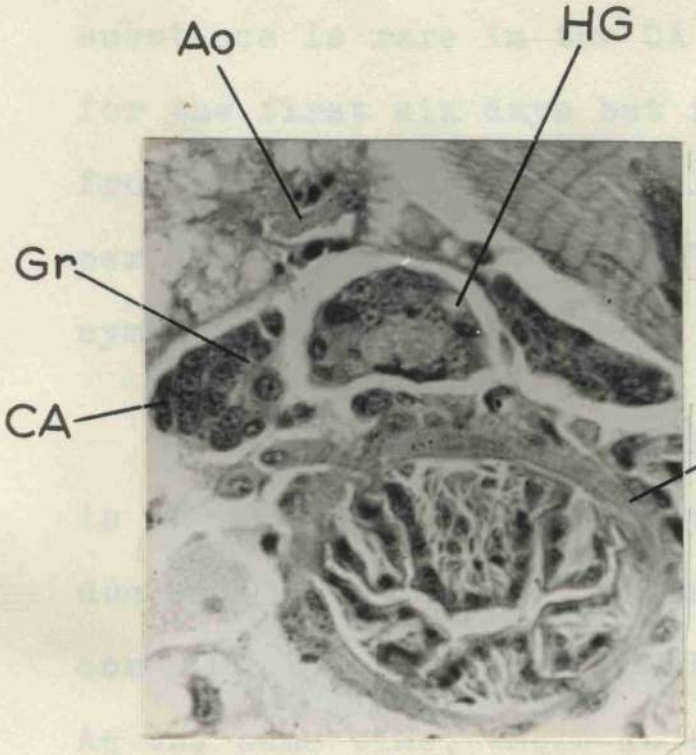


Figure 67.

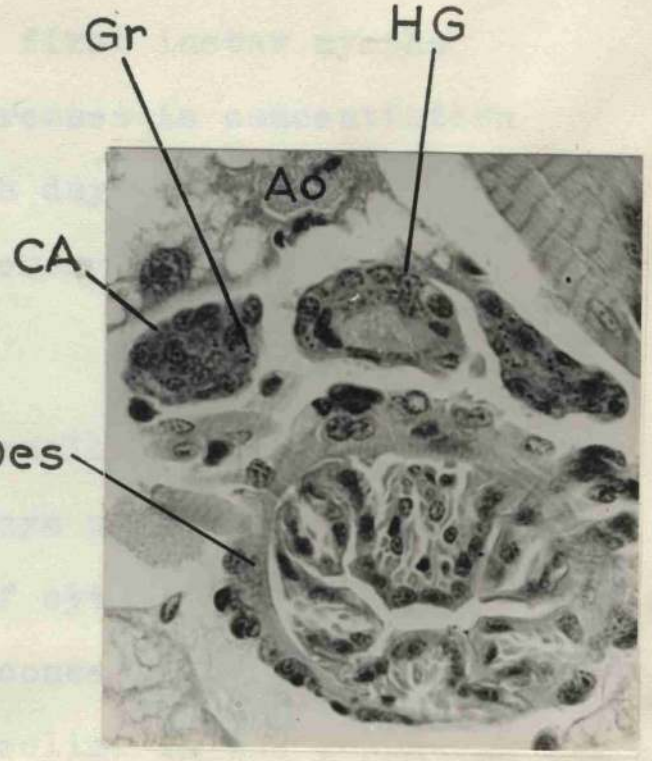


Figure 68.

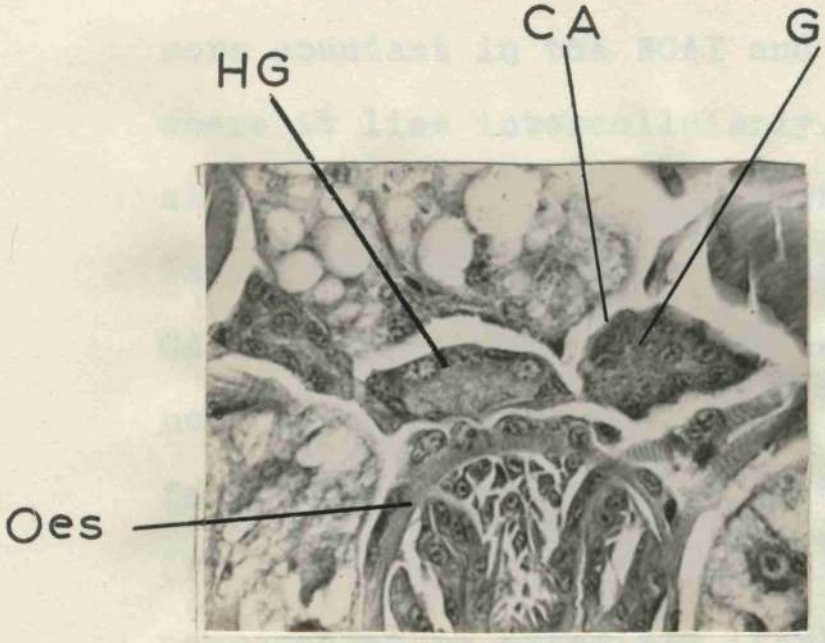


Figure 69.

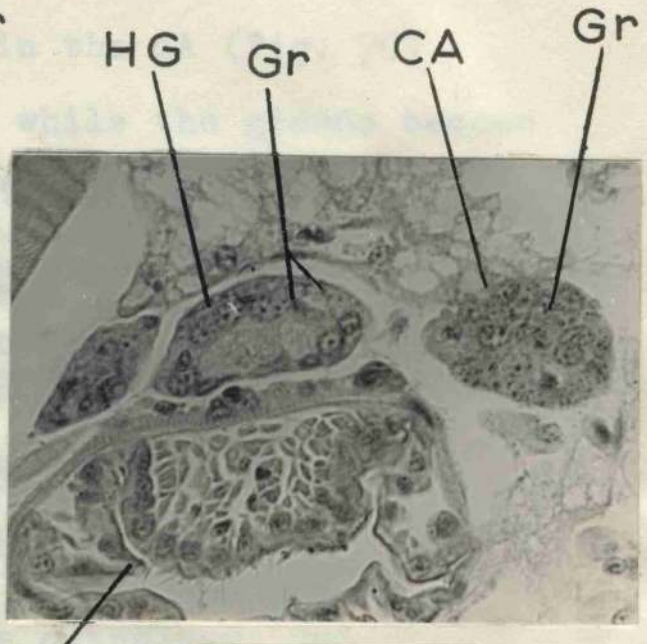


Figure 70.

of the 42-day embryo have more cytoplasm and a lower nuclear density than in later embryos. Neurosecretory substance is rare in the CA of first instar nymphs for the first six days but increases in concentration from the seventh to the twelfth day (Figs 67, 68, 69), persisting in the CA of newly emerged second instar nymphs.

The second instar CA are small at first but there is an increase in size a few days after the moult, due to an increase in volume of cytoplasm with a corresponding decrease in the concentration of nuclei. At the same time, there is a decline in the amount of neurosecretory product present. A few days before the second moult the neurosecretory material is again more abundant in the NCAI and in the CA (Fig. 70) where it lies intercellularly, while the glands become slightly reduced in size. This pattern is repeated in later nymphal instars. Of special interest are the CA of freshly moulted last instar nymphs killed one hour after moulting (Fig. 71), and those of adult females sacrificed less than one hour after moulting (Figs 72, 73). These are small, have abundant neurosecretory material, little cytoplasm, and concentrated small nuclei. But the CA of a last

Figure 71. Photomicrograph of the frontal section of the corpus allatum (CA) of a last instar ♀ nymph of P. americana, 1 hour after the moult, showing abundant PF-positive neurosecretory granules (Gr). Note also the high nuclear density and low cyto-:plasmic volume. Note the axons in the central part of the corpus allatum. Compare with figure 74 which is at the same magnification.

Figure 72. Photomicrograph of the frontal section of the corpus allatum (CA) of an adult ♀ P. americana (within 1 hour after moult), showing abundant PF-positive neurosecretory granules (Gr), high nuclear density and low cytoplasmic volume.

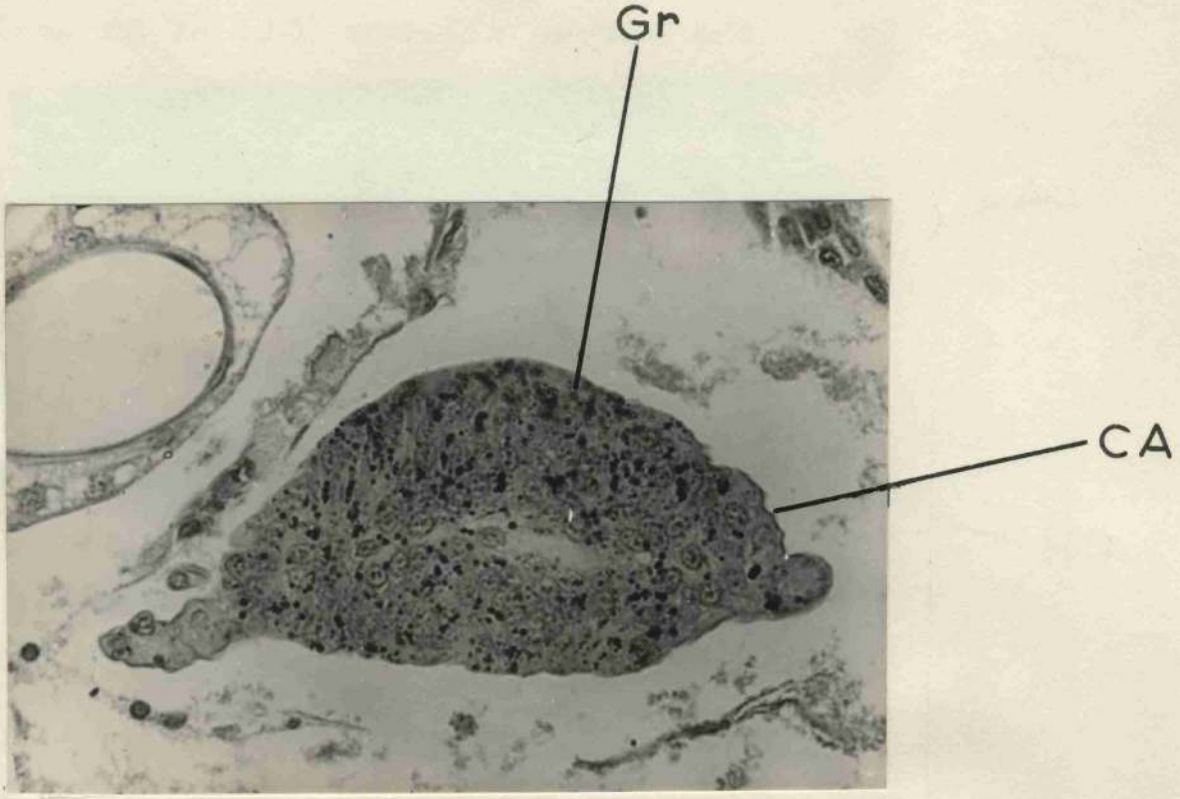


Figure 71.

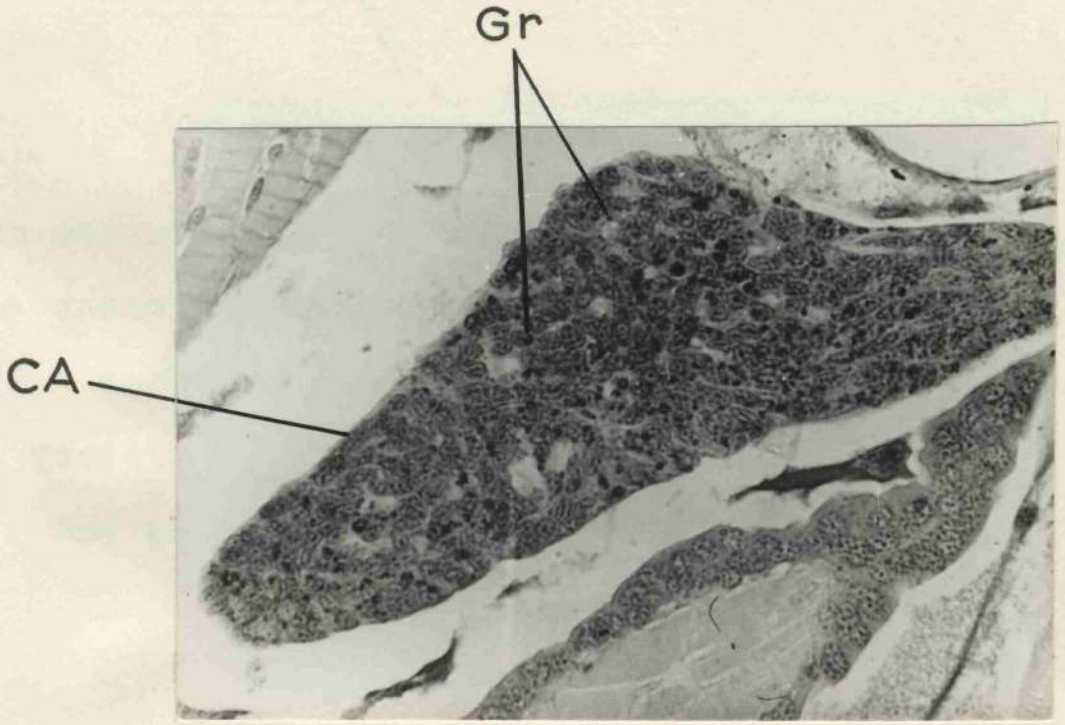


Figure 72.

Figure 73. Photomicrograph of the frontal section of the corpus allatum (CA) of an adult ♀ P. americana (within 1 hour after moult), showing abundant PF-positive neurosecretory granules (Gr). Hg, hypocerebral ganglion; Ao, aorta; note also neurosecretory granules at the periphery of the hypo-:cerebral ganglion. Same section as shown in Figure 72 but at a lower magnification.
Compare with figure 75 which is at the same magnification.

Figure 74. Photomicrograph of the frontal section of the corpus allatum (CA) of last instar ♀ nymph of P. americana, 9 hours after moulting, showing sparse neurosecretory granules (Gr), low nuclear density and high cytoplasmic volume.

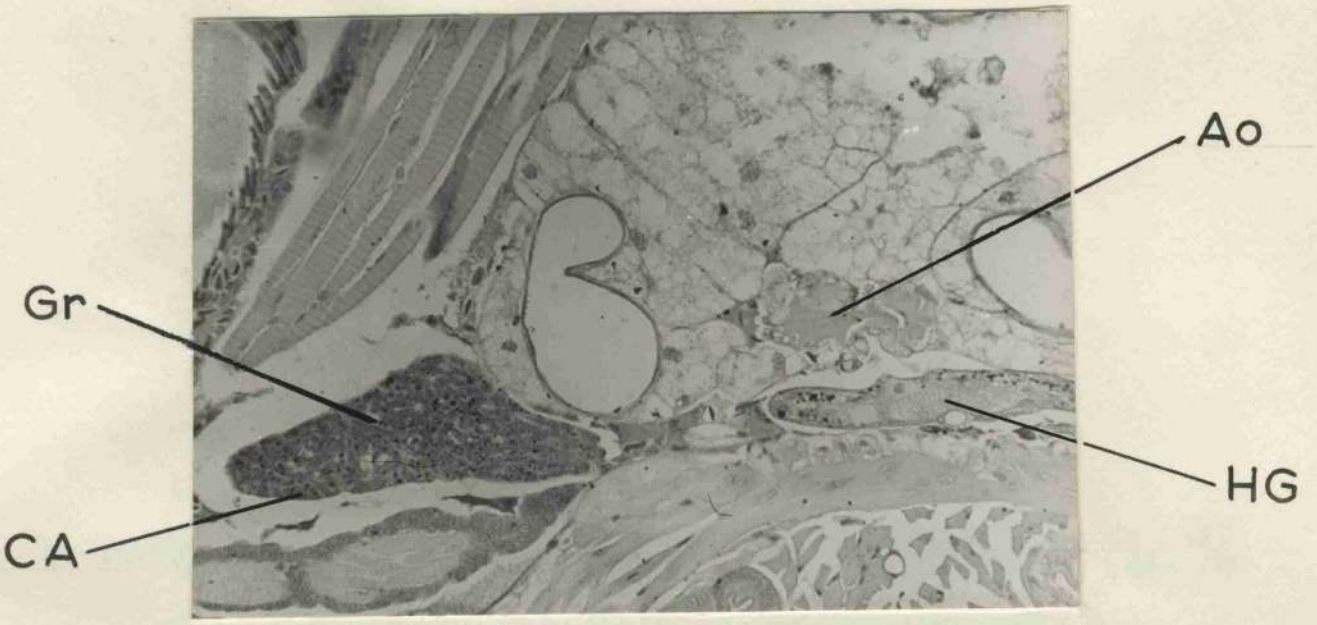


Figure 73.

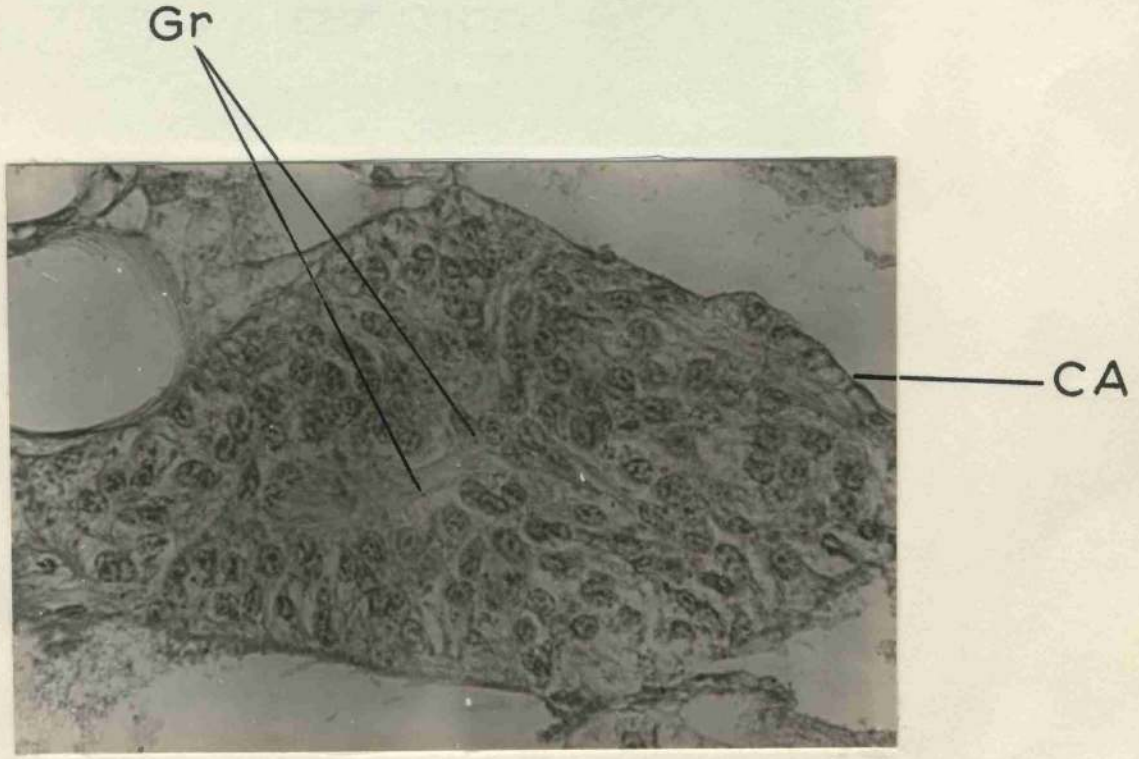


Figure 74.

Figure 75. Photomicrograph of the frontal section of the corpus allatum (CA) of an adult ♀ P. americana, killed more than 1 hour after the moult, showing sparse neurosecretory granules (Gr), Hg, hypocerebral ganglion.

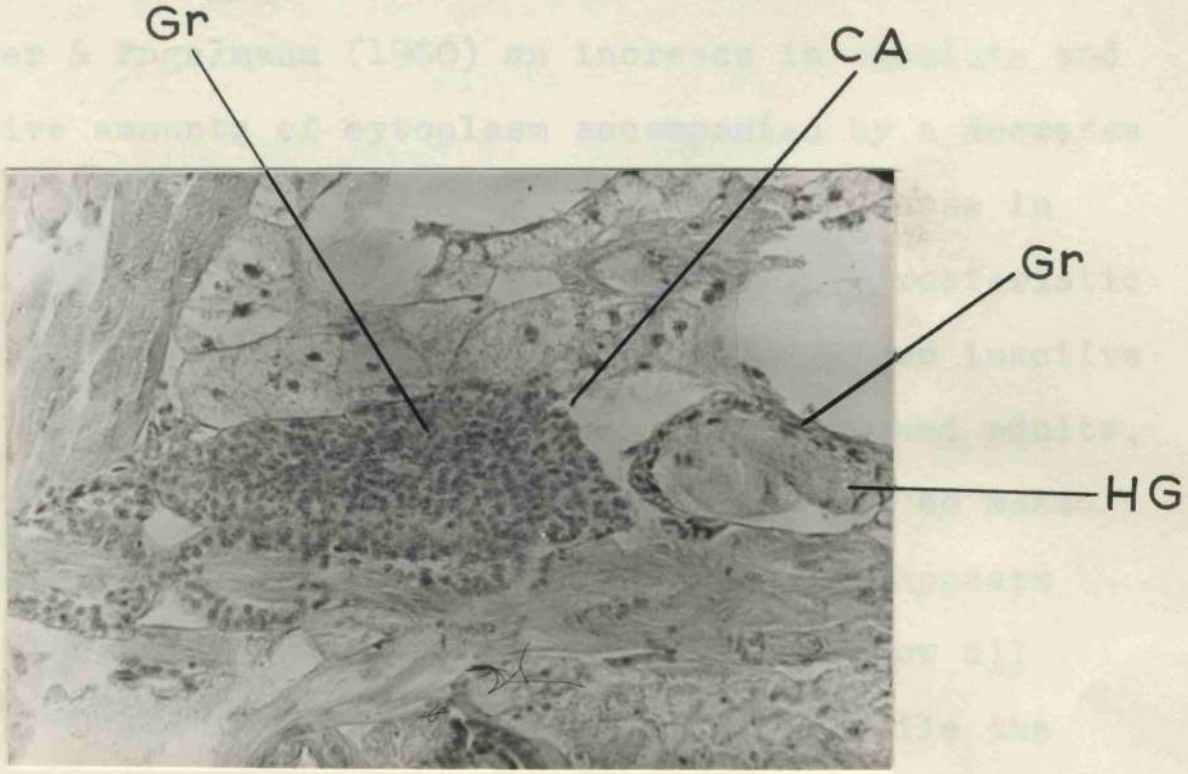


Figure 75.

instar nymph 9 hours after the moult (Fig. 74) and similarly the CA of an adult female, more than 1 hour after the moult (Fig. 75), are large and contain sparse neurosecretory material, non-aggregated and bigger nuclei, and abundant cytoplasm. According to Scharrer (1952a,b, 1958, 1961), Engelmann (1957) and Lüscher & Engelmann (1960) an increase in absolute and relative amounts of cytoplasm accompanied by a decrease in the concentration of the nuclei, and increase in the nuclear diamter (Scharrer, 1961) are characteristic of an active CA, the reverse being true for an inactive CA. Thus the CA of ffeshly moulted nymphs and adults, described just now, are inactive but as soon as most of the stainable neurosecretory material disappears from the CA of these animals these glands show all the characteristics of an active gland. While the neurosecretory substance is abundant in the CA of freshly moulted last instar nymphs and adults it is rare in the CA of sexually mature adults. The CA of the adult female gradually increase in size after the last moult. The CA of adult females 15 days after moulting are definitely larger than those of 72-hour individuals. The CA of mating individuals are also notably enlarged, the CA of the female being much larger than those of the male.

Section V: Suboesophageal Ganglion

The first sign of neurosecretion in neurons of the SOG of P. americana occurs in the 42-day embryo and can be seen subsequently in the 50 and 54-day embryos and at some time in every subsequent instar. In any one specimen some cells will show a varying degree of pink or faint purple staining of cytoplasm while in others PF^+ granules are present in varying amounts. If a cell with a definite location is observed in a large number of specimens it can be seen that at one time, the cytoplasm stains a uniform pink to light purple colour, while at another there are only a few pink to light purple granules and vacuoles in addition to the same colour of cytoplasm. Again, at another time, the same neruosecretory cell may be loaded with dark purple granules or small aggregates of granules. The appearance of any NSC at a given time probably depends on the stage in the secretory cycle at which it was fixed as well as on the chemical nature of its product. Sometimes fine PF^+ granules were also observed in nerve fibres in the antero-median and ventro-posterior part of the neuropile in this ganglion. (For location of NSC in this ganglion see Chapter 4.)

Figure 76. Photomicrograph of frontal section of brain of a freshly moulted nymph of B. orientalis, showing a small neurosecretory cell (NSC) lying antero-lateral to the internal calyx (Int.CX) and in between the lateral globuli cells (LGC). This neruosecretory cell has probably been recruited from the lateral globuli cells.

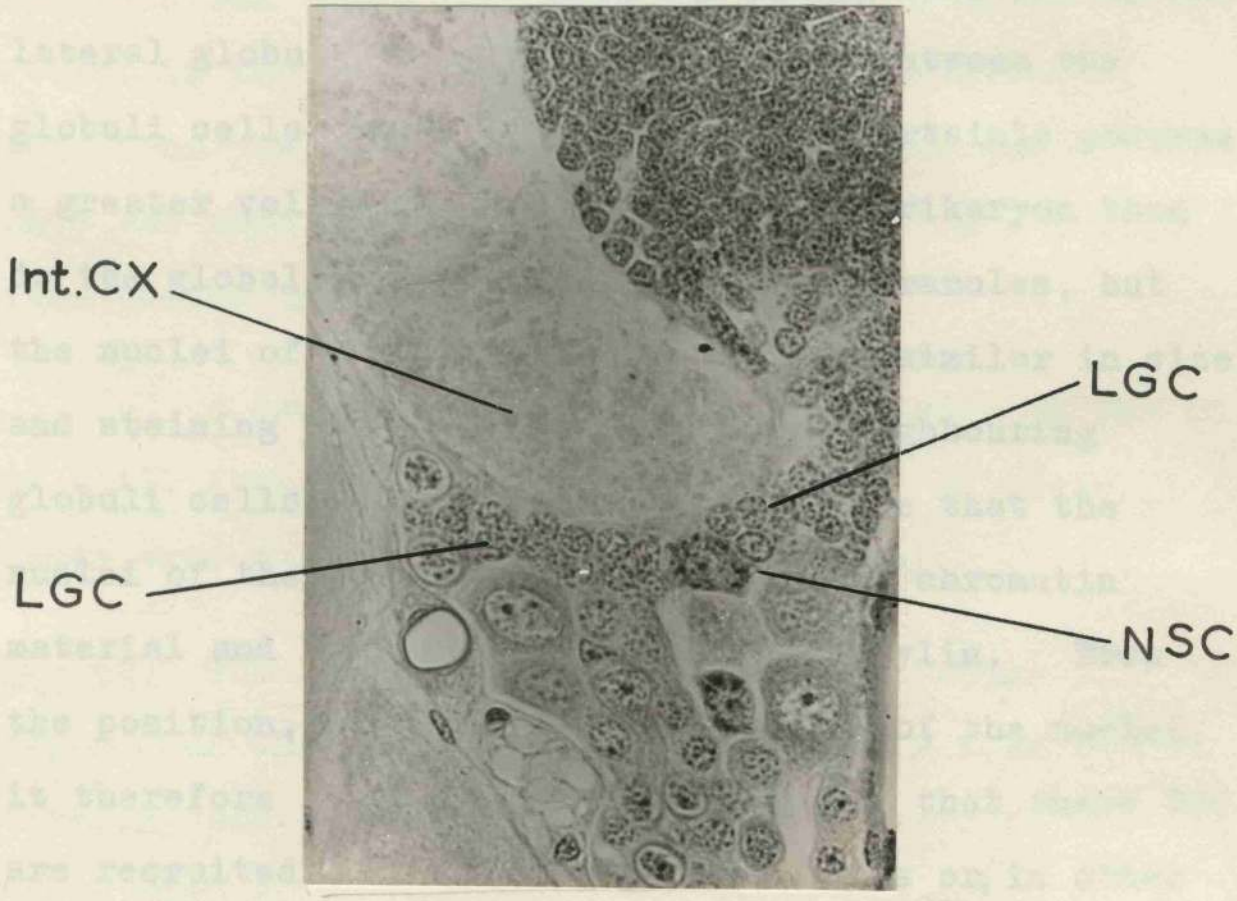


Figure 76.

Section VI: Recruitment of NSC from Globuli Cells

Small NSC occur towards the anterior limit of the median pars intercerebralis, close to, and at the border of, the lateral group of globuli cells of the internal calyx of the corpus pedunculatum. These NSC can sometimes be seen lying in the same row as the lateral globuli cells and occuring in between the globuli cells (Fig. 76). These NSC certainly possess a greater volume of cytoplasm in the perikaryon than do the globuli cells, and contain PF^+ granules, but the nuclei of these NSC are remarkably similar in size and staining properties to those of neighbouring globuli cells. It can be recalled here that the nuclei of the globuli cells are rich in chromatin material and stain darkly with haematoxylin. From the position, size, and the similarity of the nuclei, it therefore seems reasonable to assume that these NSC are recruited from lateral globuli cells or, in other words, some of the lateral globuli cells acquire more cytoplasm and become NSC,

Section VII: A Histological Investigation of an Abnormal 29-day-old First Instar Nymph

In a batch of 15 first instar nymphs of P. americana

moulting occurred 12½-13 days after eclosion but one exceptional individual which failed to moult was killed after 29 days. A histological examination of its brain revealed the following facts:

(a) The level of meristematic activity in the brain was quite low, only six dividing cells being present in the whole brain in the four formation centres of the globuli cells of the corpora pedunculata. There are about 30-40 such dividing cells in a normal first instar nymph.

(b) A few PF^+ granules were to be found in the CC and still fewer in the CA, and the nuclei of these glands were noticeably smaller than normal.

(c) The size of the CC and the CA, though bigger than that of a first instar nymph ½ hour after hatching, was smaller than those of 6-8 days first instar nymphs.

(d) There was not a single PF^+ neuron in the protocerebrum.

(e) No neurosecretory material was seen in transit in the NCC, and it must be assumed that the sparse neurosecretory material present in the CC must

have been reserves of material transmitted from the brain NSC some time previously.

It is evident, that, in this abnormal nymph, the brain NSC which normally stimulate directly or indirectly, processes of growth and development were not functioning.

DISCUSSION

The NSC are found in the brain of the embryo of P. americana as early as 42 days. The suggestion of Füller (1960) that brain NSC do not start their secretory activity, in P. americana, until the second instar, and his consequent assumption that the neurosecretory material occurring earlier in the CC is formed in loco, must be refuted.

Large peripheral cells in the embryonic protocere-:brum, described as neurosecretory cells in the locust embryo by Jones (1956), and in the embryo of Dysdercus by Sharan & Sahani (1960), certainly occur in the brain of the embryo of P. americana at a time when neurons have not become differentiated in that location. These large cells can be identified as neuroblasts, some of which contain an inner layer of cytoplasm which is moderately PF^+ but these neuroblasts do not, of course,

possess axons nor does neurosecretory material occur in the developing CC at this time. In the mature larva of Musca domestica Panov (1957) described similar large vacuolated peripheral cells in the protocerebrum, which he, too, identified as neuroblasts.

At the forty-second day of embryonic life the brain has developed a structure which is morphologically similar to that of the early nymphal instars, and which subsequently remains essentially the same throughout the insect's life (see Chapter 2 above). After this stage the only changes which occur are a steady increase in number and cytoplasmic volume of the neurons, a gradual increase in size of the neuropile and an increase in the number of glia cells and NSC of the protocerebrum.

There are indications that neurons of particular types, or groups, function at equivalent times in successive instars. According to their location, the NSC of the protocerebrum of both species of cockroaches fall into three major groups: median NSC whose axons characteristically cross to the opposite lobe before leaving the brain, lateral NSC whose axons characteristically take a direct exit course, and ventral NSC whose axons are now known to join those of the median NSC to form

the NCCI (Willey, 1961). The occurrence of lateral and ventral NSC in P. americana and B. orientalis is recorded here for the first time. These three NSC groups can be demonstrated by the staining procedures already described, at some time in all stages of development, from the 42-day embryo to the sexually mature adult, as can the NSC of the suboesophageal ganglion.

The PF^{+} material in NSC of embryos and newly hatched first instar nymphs does not appear in the same abundance as in NSC of later instars. The granules are small, fine, and dispersed and must be sought with care.

The fact that in any given cell the stainable substance is scarce may indicate either a low rate of production or a high rate of production conjoined with a high rate of discharge. NSC in last instar nymphs and adults interpreted by Füller (1960) as very active cells, may either be cells in which the rate of accumulation of product exceeds the rate of discharge or cells in which the product has previously accumulated to be discharged later. In the past it was often assumed that a NSC loaded with neurosecretory material was in a very active state, the reverse being true for

an inactive cell. At the third internal symposium on neurosecretion (Bristol, 1961), E. Scharrer and others said that a NSC loaded with neurosecretory material is in a resting stage while a small amount of neurosecretory material indicates a high secretory activity. Highnam supported this view with experimental evidence, from work involving the use of radioactive tracers. E. Scharrer suggested that the accumulation of neurosecretory material in the perikaryon depresses the rate of cytoplasmic synthesis and so forms a self-limiting mechanism for the production of neurosecretion. In modern terminology this may be known as 'intra-cytoplasmic feed back'.

In the CC there is a cycle of increase and decrease in size in successive instars, due to increase and decrease in the amount of neurosecretory material stored in the swollen termini of the axons of the NCC. In the first and second instars at least, the CC are actually slightly decreasing in volume at times when the flow of neurosecretory product from the brain, as indicated by the amount detectable in transit in the NCC, is increasing. Thus changes in volume of the CC appear to be related to the storage and release function of these glands and not solely to the level

of neurosecretory activity of the brain cells. It seems probable that the products of those brain NSC which are most active before the CC attain maximum size in a given instar are stored before release, while the products of NSC which discharge later in the instar pass quite rapidly through the CC into the blood. It must be emphasised that the first such cycle occurs, not in the first instar as might be expected, but in the late embryo prior to eclosion.

The presence of abundant neurosecretory material in the CA of final instar nymphs and adults, which have just moulted, is presumably related to the restraining action of brain NSC on the CA demonstrated experimentally by B. Scharrer (1946c, 1952b, 1961), but never before confirmed by histological evidence. This neurosecretory material is also present in the nervi corporis allatai I (NCAI), in the nervi corporis allati II (NCAII) and in the commissure of the corpora allata (CCA), and when present in abundance in these it forms aggregates of various sizes in addition to the small scattered granules.

The presence of these intercellular neurosecretory products in the CA in large amounts together with the decline in volume at nymphal moults, indicates that the CA are to some degree restrained at every moult. The

relaxation of this restraint, indicated by a decrease in the amount of neurosecretory product in the CA, is accompanied by an appreciable increase in the size of the gland which has now a large volume of cytoplasm and larger and less concentrated nuclei. There is histological evidence of such restraint being applied in the late embryo prior to eclosion. It may well be that the small amount of neurosecretory material reaching the CA perhaps stimulates the latter to produce a certain amount of juvenile hormone for a certain time; and then as the time for moulting approaches, the activity of the CA which has to be restrained is checked by the overflow and consequent presence of abundant neurosecretory material in these glands. The other possibility is that the presence of small amounts of neurosecretory material in the CA does not stimulate the glands but just allows the CA to do a certain degree of work. They are allowed to produce a certain amount of juvenile hormone under the control and supervision of brain NSC (which may not be working completely independently of the CA) for a certain period after which the overflow and accumulation of neurosecretory material in the CA make these glands inactive for some time. It should be noted here that the rate of change of the CA is very

rapid in newly moulted last instar nymphs and adults, and so in order to observe the histological changes in the CA one should kill the animals at short intervals of time.

It is now generally accepted that the cycle of changes in the neuroendocrine system which is repeated in successive nymphal instars is related to the control of development and initiation of moulting. That such a cycle occurs first in the embryo before eclosion indicates that there is a physiological similarity between the processes of eclosion and of moulting. The existence of a capillary system either around the brain cells or in the CC as described by Grandori (1954, 1955) in adult flies (Calliphora and Musca) cannot be confirmed in the two species of cockroach studied here. When the neurosecretory product passes from the CC into the lumen of the aorta it is diluted and is no longer stainable, though in addition to the diffuse, moderately PF^+ coloration of the aorta wall sometimes a few PF^+ granules were observed, and these may have been neurosecretory in origin. Nayar (1956) and Johansson (1958) have also reported the presence of neurosecretory material in the aorta of Iphita and Oncopeltus respectively. The presence of haemocytes with PF^+ granules were noted in the aorta of the cockroaches.

CHAPTER 4

NEUROSECRETORY CELLS IN THE BRAIN, FRONTAL GANGLION, AND SUBOESOPHAGEAL GANGLION OF *PERIPLANETA AMERICANA* L. AND *BLATTA ORIENTALIS* L.

INTRODUCTION

Fraser (1957) demonstrated that in the brain of the larva of the blowfly, *Lucilia*, no fewer than six groups of neurosecretory cells (NSC) can be detected. He grouped these NSC on the basis of their position, size, morphology, staining characteristics and times of activity. The aims of the present investigation were, firstly, to determine whether it is possible to classify the NSC of the brains of the cockroaches, *Periplaneta americana* L. and *Blatta orientalis* L., in groups or cell types on the basis of similar criteria and, secondly, to determine whether NSC occur in the brain in locations other than the pars intercerebralis.

Large neurons containing stainable granules were reported, by Bretschneider (1914), in the pars intercerebralis of *B. orientalis* but, of course, at that time the phenomenon of neurosecretion was unknown and the first actual description of NSC in either of the species in question is attributable to Scharrer (1941),

who found signs of neurosecretory activity in nerve cells in the median pars intercerebralis of three out of twenty-two specimens of P. americana which she studied. She used what was at that time the standard stain for neurosecretory material, namely Foot's modifications of Masson's trichrome and, as has been learnt subsequently, this stain selectively colours the larger droplets in certain types of cells only (Scharrer & Scharrer, 1954b). The inadequacy of the original staining procedure led her to the erroneous conclusion that there are far more NSC in the suboesophageal ganglion than in the brain and that the median pars intercerebralis of cockroaches is of secondary importance as far as the neurosecretory activity is concerned. She further stated that NSC are among the largest cells found in the central nervous system, but in a later study on Leucophaea, Scharrer & Scharrer (1944) found that there are varying numbers of medium-sized NSC in the pars intercerebralis of that species, these cells containing varying amounts of neurosecretory material. The presence of NSC in the median pars intercerebralis of P. americana and of B. orientalis was confirmed by Stutinsky (1952), using chrome haematoxylin phloxin stain.

Until the present work was undertaken no account had

been published of an attempt to separate the NSC of the pars intercerebralis of cockroaches into groups or cell types, lateral NSC were unknown in these insects and, in fact, the presence of NSC in any part of the brain other than median pars intercerebralis was unsuspected. However, during the course of this work three papers on this topic were published. The first, by Drescher (1960), is mainly concerned with the regeneration of structures in the brain of P. americana after injury but in this paper he also makes some observations on neurosecretion. The second, by Füller (1960), deals specifically with neurosecretion in P. americana and B. orientalis. The third, by Willey (1961), is mainly concerned with the stomodaeal nervous system of P. americana and of other cockroaches, excluding B. orientalis, but this work also contains certain observations on the neurosecretory system. Two other recent papers on Orthoptera are of importance. The first, by Highnam (1961), dealing with the histology of the neurosecretory system of the locust, Schistocerca, and the second, by Blest (1961), dealing mainly with a method of staining the nervous system of the same species.

In most recent studies of NSC the two staining procedures, chrome haematoxylin phloxin (CHP) and

paraldehyde fuchsin (PF) (see Chapter 3), have been used and as a result two types of neurosecretory cells have been distinguished, 'A' cells which have abundant secretory granules staining dark blue after CHP and purple after PF staining, and 'B' cells which either assume a uniform red colour or are found to contain sparse red granules after CHP (the red being the counterstain phloxin) and which are variously described as red, purple, or bluish green (the counterstain) after the PF procedure (e.g. Nayar, 1955a; Kopf, 1957; Kobayashi, 1957; Johansson, 1957, 1958; Ozeki, 1958b; Highnam, 1961).

There is a marked difference of opinion among neurocytologists about 'B' cells. Some consider that the 'B' cell appearance represents a stage in the secretory cycle of an 'A' type cell (e.g. Thomsen, 1954a,b; Nayar, 1955a; De Lerma, 1956; Herlant-Meewis, 1956), while others regard this as a distinct cell type with a secretory cycle of its own (e.g. Kobayashi, 1957; Johansson, 1957, 1958; Highnam, 1961).

Regarding the location of NSC in the brain of orthopteroid insects most recent reports indicate that NSC are found in the pars intercerebralis only (Drescher, 1960; Füller, 1960; Highanm, 1961; Pipa, 1961b).

Füller (1960) has described only one cell type in the median pars intercerebralis of P. americana and B. orientalis. This consists of medium-sized asynchronous NSC of the 'A' type which he places in a sub-category A-α. His other sub-category A-β consists of synchronous NSC in parts of the central nervous system other than the brain. Very recently Pipa (1961b) has identified Füller's (1960) A-α cells as 'A' cells in the brain of P. americana, the only type of neurosecretory cell he describes.

Drescher (1960) indicates the existence of three cell types in the pars intercerebralis of P. americana. The first is represented by one large cell in each half of the brain, lying ventro-lateral to the inner calyx. The second, which is also large, appears only in a photomicrograph and lies ventral to the calyx, but which calyx this is, is not indicated, and the third consists of numerous smaller cells lying below the large cells.

Willey (1961) wisely avoids the issue of 'A' and 'B' cell types but uses location to identify five groups of NSC in the protocerebrum of P. americana, and other cockroaches. The axons of cells of four of his groups contribute mainly to the nervi corporis cardiaci interni (NCCI) while the axons of his group five cells and also

the axons of some of his group 4 cells contribute to the nervi corporis cardiaci externi (NCCII) (see Chapter 1). Recently he appears to have decided that his group 4 cells lying near and under the calices are not neurosecretory (personal communication). Willey's (1961) large cells of his group 4 correspond to Drescher's (1960) large NSC and, probably to the large NSC described by Highnam (1961) as lying between the pars intercerebralis and the mushroom body in Schistocerca and to a large NSC lying ventral to the calyx noted also by Blest (1961) in Schistocerca.

In adult Leucophaea, Scharrer (1955b) described one ventro-lateral and one latero-lateral NSC, in each half of the suboesophageal ganglion (SOG), packed with deep purple granules after paraldehyde fuchsin staining. These cells, which she called 'A' cells, are said to possess fewer cytoplasmic granules in nymphs than in adults. She further stated that there is another pair of 'B' cells, which are larger than the 'A' cells and contain only a few peripheral purple granules. This pair of 'B' cells (one cell in each half) she described as being more medial, slightly caudal, and in the vicinity of the ventro-lateral 'A' cells.

According to Füller (1960) the NSC of the SOG of

P. americana cannot be clasified into 'A' and 'B' types, as Scharrer (1955b) has done in Leucophaea, on the basis of the amount of neurosecretory material present in the cells. Füller described only three groups of NSC in the SOG of this species of cockroach, lateral, ventro-lateral, and ventro-medial. Further he held that all the NSC found in this ganglion are A-β type (synchronous) and that none of them has abundant neurosecretory granules. It will be seen later that Füller's description of NSC does not agree with the author's observations.

MATERIAL AND METHODS

Serial sections (6μ) of the heads of embryos, nymphs and adults of P. americana, and those of nymphs and adults of B. orientalis stained by chrome haematoxylin phloxin and paraldehyde fuchsin procedures, as described in Chapter 3, were used in this study. Material in neurons or their axons which stain blue-black after chrome haematoxylin is hereafter referred to as CH^+, material staining pink or purple-red with paraldehyde fuchsin is called PF^+.

OBSERVATIONS

Whether or not all the cells described below as neurosecretory cells, secrete hormones is not yet known. However, if cytomorphological criteria for the identification of neurosecretion are to be accepted at all, these cells which show such signs as appropriately stained granules in the perikaryon, axon-hillock and (usually) in the axon, and also cyclical actively, must, unless proved otherwise, be regarded as neurosecretory cells. For a fuller definition of 'neurosecretory cell' see Chapter 3.

Section I: Neurosecretory Cells in the Brain

1a NSC in the protocerebrum

The numerous NSC in the protocerebrum of both species of cockroach studied here occur in six definite locations.

Locus 1.

These NSC lie in the posterior pars intercerebralis, ventral to the protocerebral neuropile, above and on either side of the two nervus connectivus cells (Figs 81, 82) which, incidentally, do not contain PF^+ or CH^+ granules. Thus they form an arch over the nervus connectivus cells but are separated from them by glia

Figure 77. Photomicrograph of a section of anterior part of protocerebrum of an adult ♀ P. americana killed less than 1 hour after the moult, showing two ventro-median (1b) neurosecretory cells which have aggregated PF^+ granules (Gr) in the perikaryon.

Figure 78. Photomicrograph of a section of anterior part of protocerebrum of an adult ♀ P. americana killed 12 hours after the moult, showing two ventro-median (1b) neurosecretory cells which have a few PF^+ granules (Gr) in their abaxonal region. Tr, trachea; NpPRC, neuropile of the protocerebrum. Figure 78 is at a lower magnification than Figure 77.

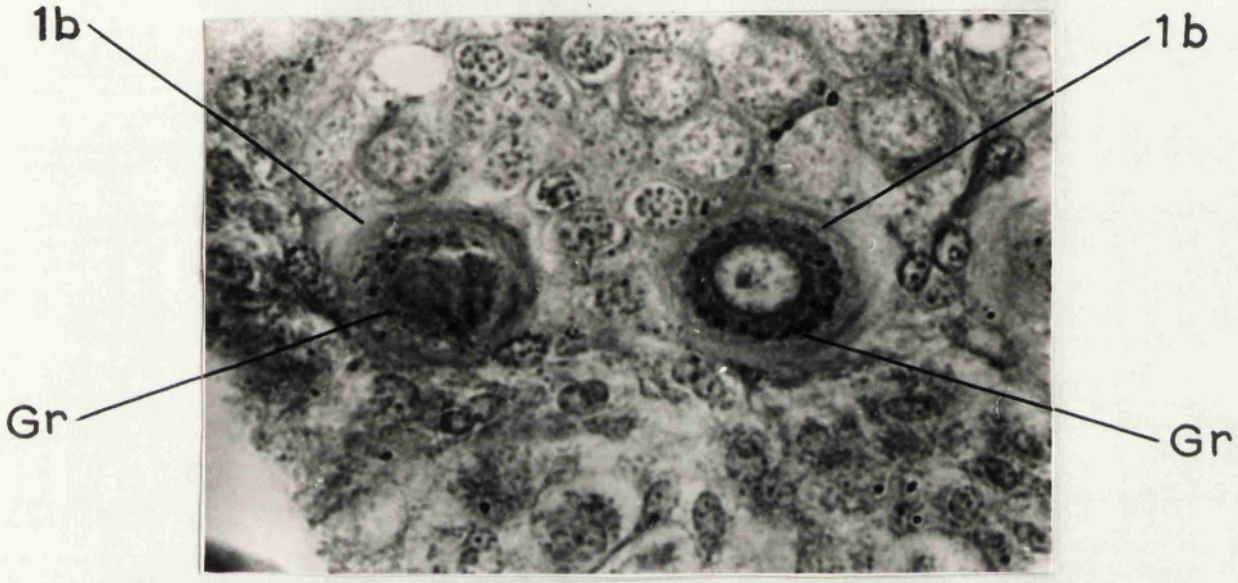


Figure 77.

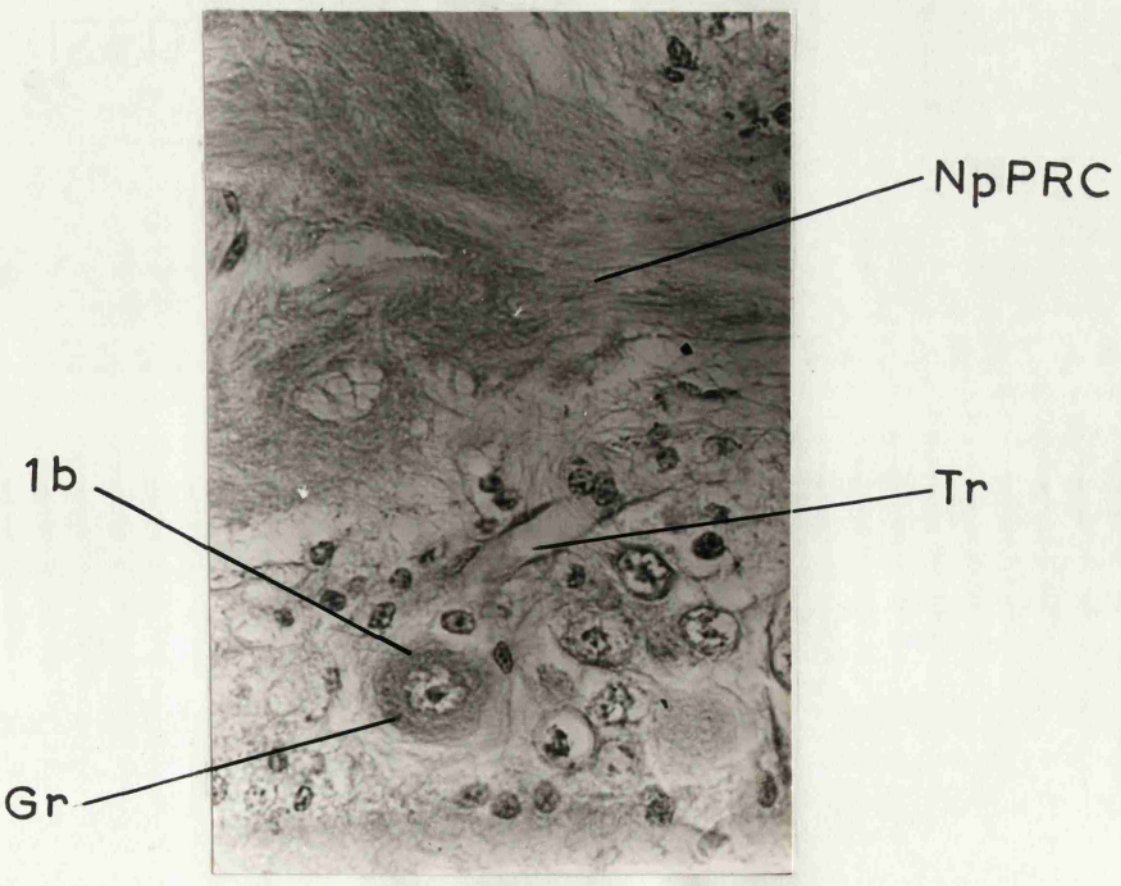


Figure 78.

Figure 79. Photomicrograph of a section of anterior part of protocerebrum of an adult ♀ P. americana, killed 18 hours after the moult, showing ventro-median neurosecretory cells (1a) of locus 1, neurosecretory cells of locus 2 and neurosecretory cells of locus 3.

Figure 80. Photomicrograph of a section of anterior part of protocerebrum of an adult ♀ P. americana, killed 18 hours after the moult, showing ventro-median neurosecretory cells (1a and 1b) of locus 1, and the neurosecretory cells of locus 3. This section immediately follows the one shown in Figure 79.

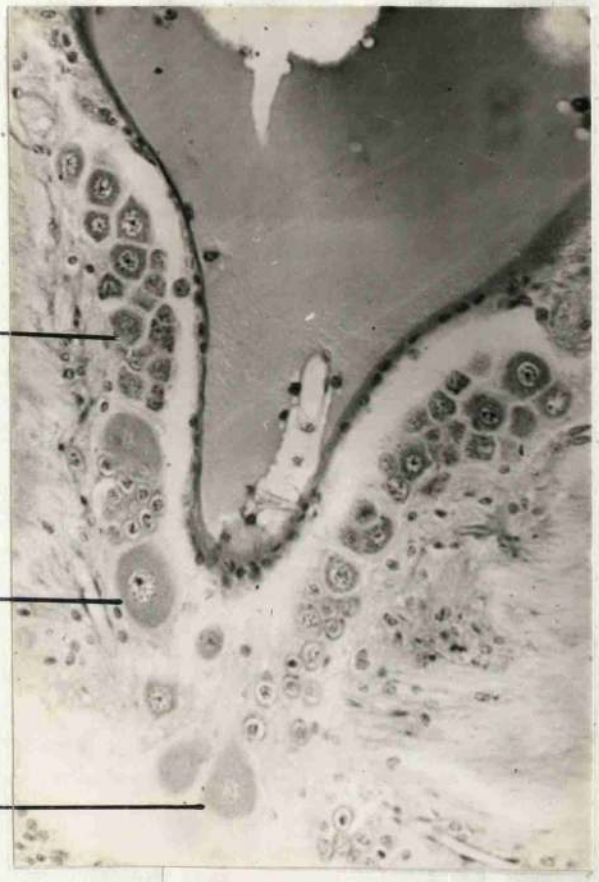


Figure 79.

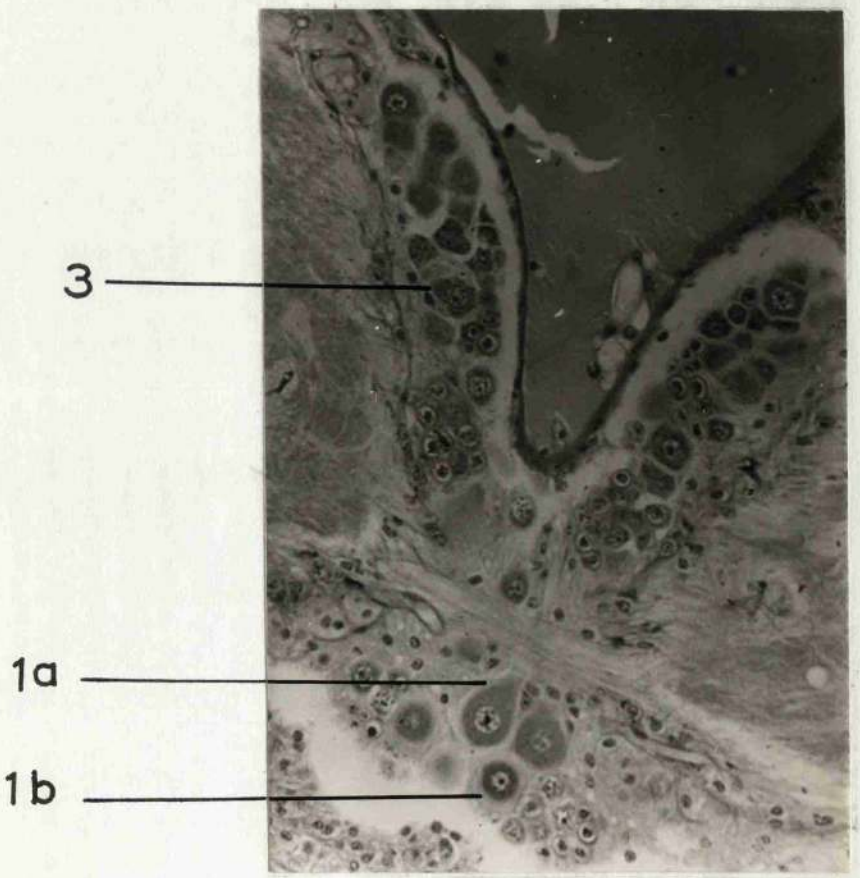


Figure 80.

cells. This region of the brain is richly supplied with trachea and tracheoles. Secretory material is probably most abundant in freshly moulted adults of both sexes (Fig. 77). In the latter the fine PF^+ granules are distributed close to the periphery of the cells and around the nucleus where they form small separate aggregates, although fine dispersed granules may also be found in other parts of the perikaryon. While the individual cells contain comparable amounts of neurosecretory material in one adult, the amounts present in different adults of the same age varies. Following the last moult the amount of PF^+ substance in these cells progressively decreases, the decrease being most rapid in the first few hours (Fig. 78), though a few granules may persist for some time after the moult. In the first stages of decrease the granules, which previously formed a peripheral ring, are now confined to a crescent shaped mass in the abaxonal zone of each cell. Ultimately, only a few scattered peripheral granules and small vacuoles can be seen.

In this locus there are two large pear-shaped antero-ventral cells (1a) lying on either side of the mid-line just below that region of the neuropile which connects the two halves of the protocerebrum (Figs 79, 80, 81, 82). Thus one such cell occurs in each half

Figure 81. Photomicrograph of a section of anterior part of protocerebrum of an adult ♀ P. americana, killed 18 hours after the moult, showing ventro-median neurosecretory cells (1a and 1b) of locus 1, two nervus connectivus neurons (NCN) the axons of which are penetrating the neural lamella (NL) of the protocerebrum. Note also the glia cells (GC) between the neurosecretory cells of locus 1 and the nervus connectivus neurons. The empty space is an artefact.

Figure 82. Photomicrograph of a section of anterior part of protocerebrum of an adult ♀ P. americana, killed 18 hours after the moult, showing ventro-median neurosecretory cells (1a and 1b), ventro-lateral neurosecretory cells (1c) of locus 1, and nervus connectivus neurons. Lettering as in Figure 81. The central space is, again, an artefact.

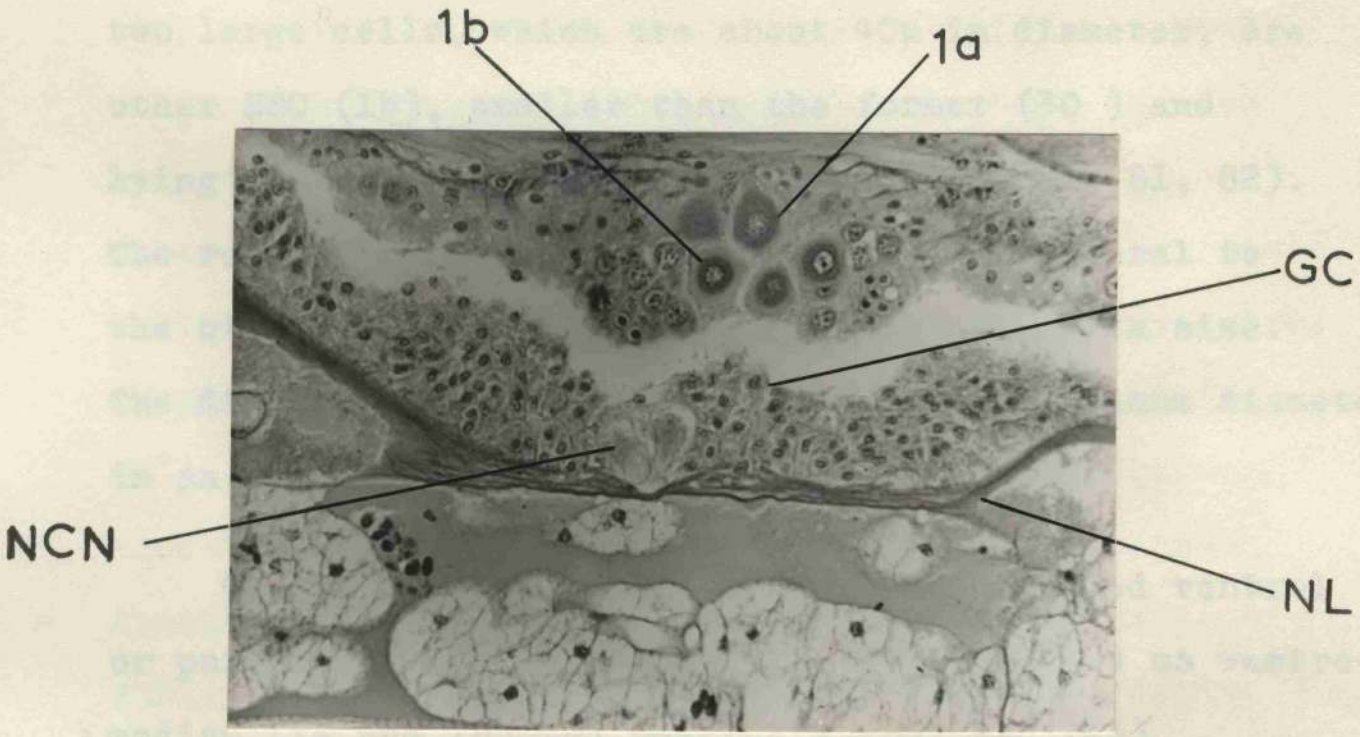


Figure 81.

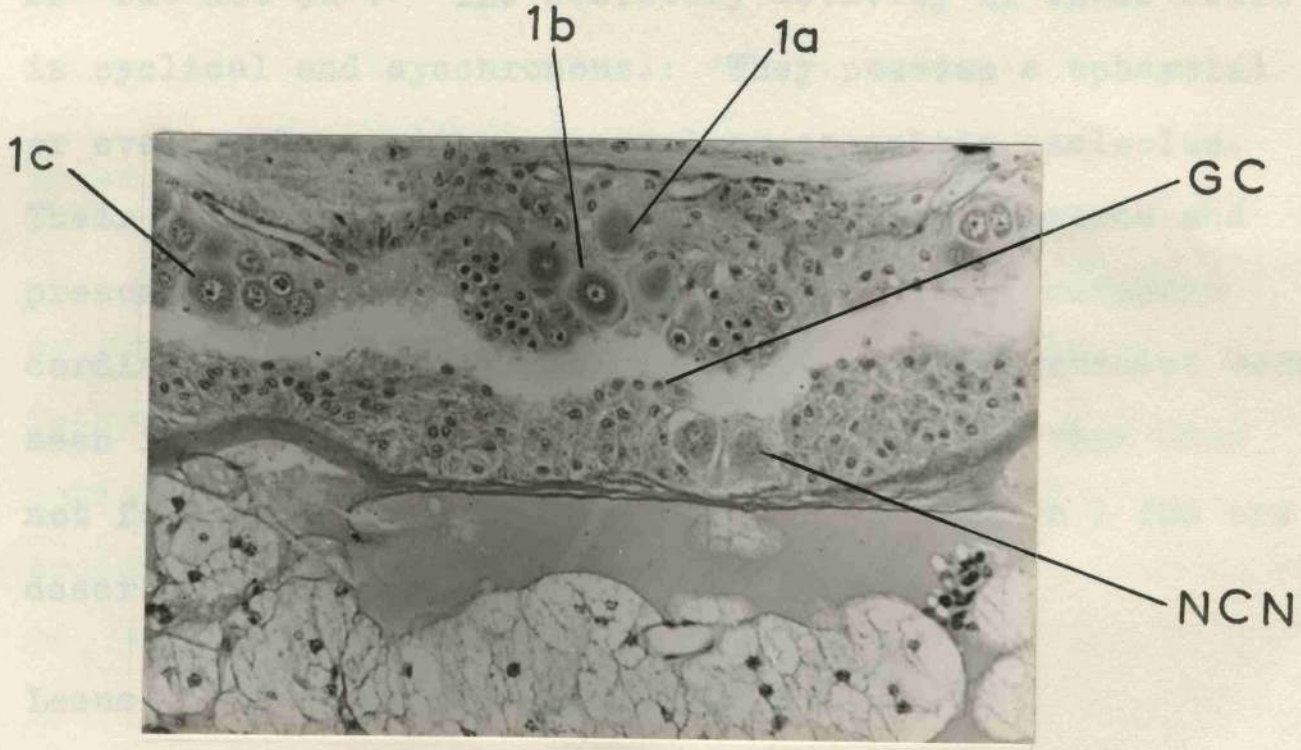


Figure 82.

of the protocerebrum. Below and by the side of these two large cells, which are about 40μ in diameter, are other NSC (1b), smaller than the former (30) and lying near the median line (Figs 77, 78, 80, 81, 82). The remaining NSC of this group (1c) lie lateral to the others (Fig. 82) and are also about 30μ in size. The dimension in each case refers to the maximum diameter in an adult.

The group 1 cells may therefore be called ventral or para connectivus NSC and may be subdivided as ventro-median (1a and 1b) and ventro-lateral NSC (1c).

The neurosecretory granules in all these cells are PF^+ but not CH^+. The secretory activity of these cells is cyclical and synchronous. They possess a sphercial or oval nucleus with a central or eccentric nucleolus. Their axons are thick and run upwards and forwards and presumably join with the prechiasmatic nervi corporis cardiaci interni (NCCI). Although the PF^+ granules were seen in the perikaryon and the axon-hillock, they were not found in the axons of locus 1 NSC. Locus 1 NSC are described as the ventral NSC in Chapter 3.

Locus 2.

NSC belonging to locus 2 (Figs 79 and 88) are

found in the anterior median pars intercerebralis. They consist of two or three large pyriform or oval neurosecretory cells (about 40μ in diamter) in each half of the protocerebrum, always lying lateral and close to the dorsal groove. After PF staining, their cytoplasm is coloured light red or sometimes grey, the grey colour being the counterstain. A few PF^+ (or CH^+) granules can be demonstrated in the perikaryon, axon-hillock and axons of these NSC. Their axons travel vertically downwards to join the prechiasmatic NCCI. The neurosecretory material of these cells is certainly transported along the axons. Their activity is cyclical and synchronous. Willey's (1961) description of NSC in this location in P. americana nymphs and adults essentially agrees with the foregoing.

Locus 3.

Locus 3 may be defined as that zone bounded anteriorly by the lower limit of the lateral globuli cells of the internal calyx, laterally by the perineurium of the dorsal groove on one side and by the main mass of the protocerebral neuropile on the other (Figs 83, 84, 85, 86, 87) and, posteriorly, by locus 2 (Figs 79 and 88), and locus 4 NSC.

The numerous NSC of locus 3 lie antero-lateral to

Figure 83. Photomicrograph of a frontal section of protocerebrum of an adult♀ P. americana, killed 18 hours after the moult, showing 3a, 3b and 3c neurosecretory cells (NSC) of locus 3. P, perineurium cell of dorsal groove; LGC, lateral globuli cells of the corpus pedunculatum; PRCNp, protocerebral neuropile; Gr, PF^+ neurosecretory granules.

Figure 84. Photomicrograph of a frontal section of protocerebrum of an adult ♀ P. americana, killed 12 hours after the moult, showing neurosecretory cells of locus 3. Note a 3c NSC lying just below the lateral globuli cells. IntCX, internal calyx. Other lettering as in Figure 83.

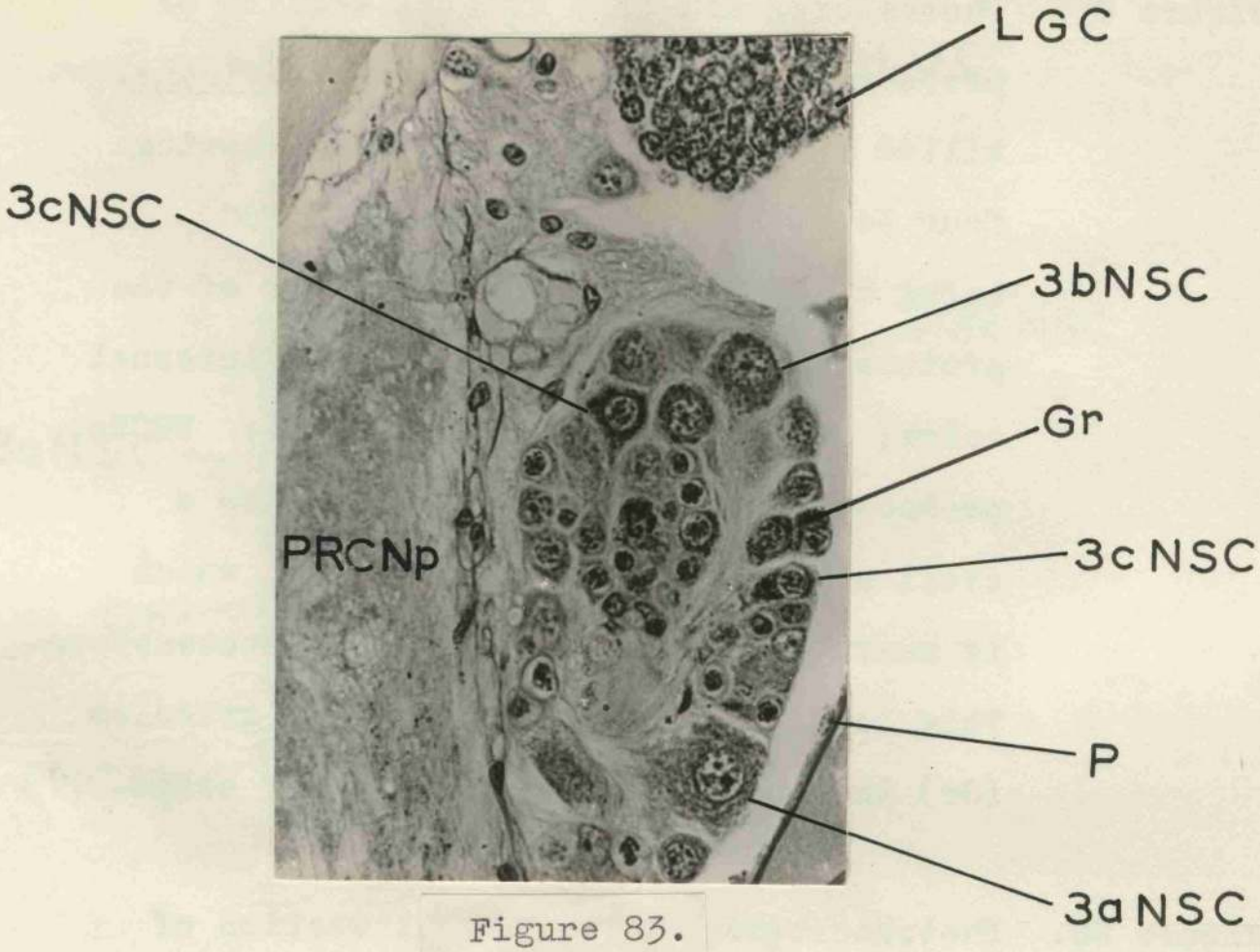


Figure 83.

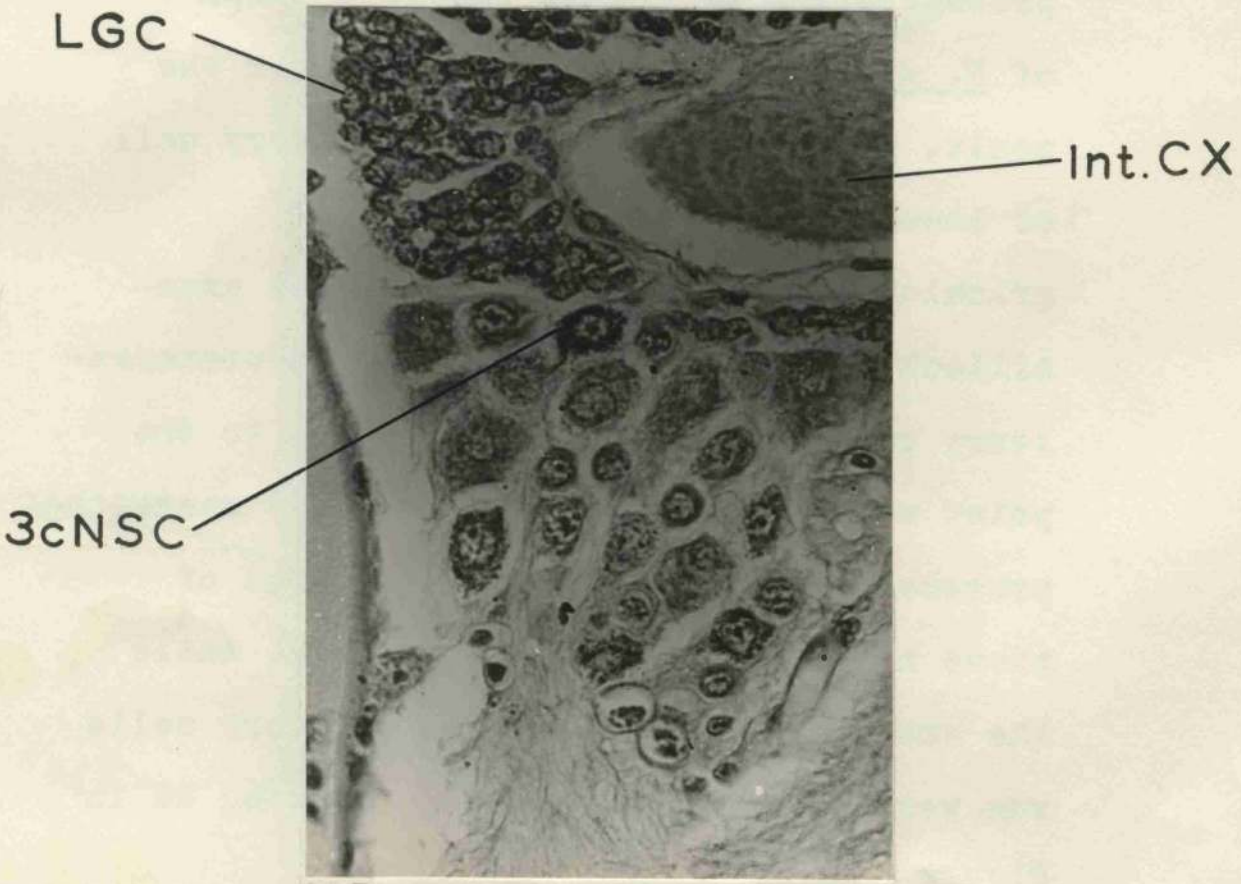


Figure 84.

Figure 85. Photomicrograph of a frontal section of protocerebrum of an adult ♀ P. americana, killed 12 hours after the moult, showing four 3c neurosecretory cells (3c NSC) lying close to the anterior border of the protocerebral neuropile. IntCX, internal calyx; LGC, lateral globuli cells; PRCNp, protocerebral neuropile. Note also a small neurosecretory cell (3c NSC) which is near the median line of the protocerebrum. This cell has less neurosecretory granules (Gr) than the other neurosecretory cells.

Figure 86. Photomicrograph of a frontal section of protocerebrum of a last instar ♀ nymph of P. americana, killed 1 hour after the moult, showing a large neurosecretory cell of locus 3 (3a NSC) with sparse PF^{+} granules (Gr) in its perikaryon and axon-hillock. Note also three small neurosecre-:tory cells (3c NSC) lying lateral to the point where the median fissure (MF) meets the protocerebral neuropile. The axons of these neurons run antero-laterally, while the axons of the other neurosecretory cells run vertically downwards. Lettering as in Figure 85.

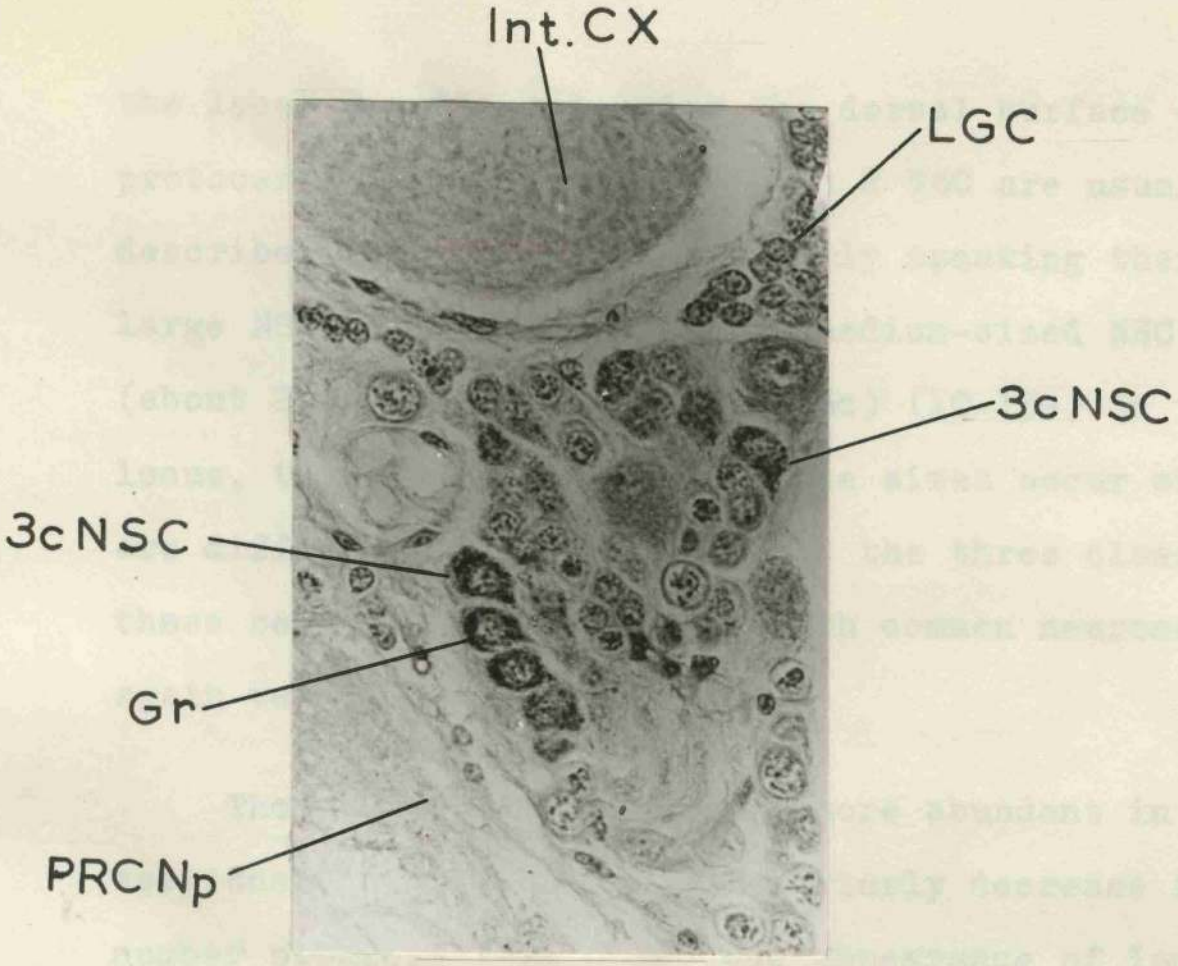


Figure 85.

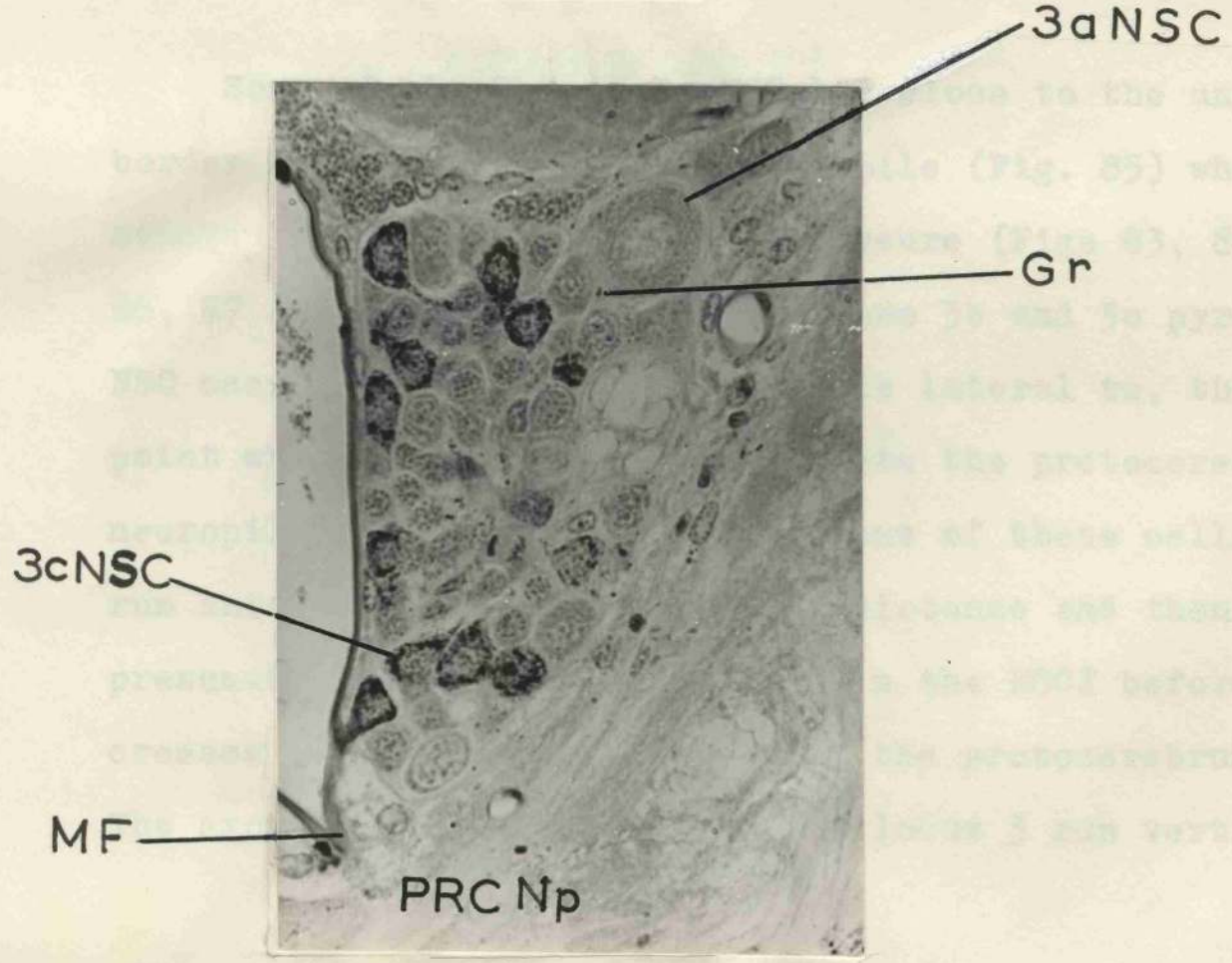


Figure 86.

the locus 2 cells and under the dorsal surface of the protocerebrum. These and locus 2 NSC are usually described as median NSC. Broadly speaking there are large NSC (3a) (about 30-40), medium-sized NSC (3b) (about 20-25μ), and small NSC (3c) (10-15μ) in this locus, though NSC of intermediate sizes occur which are difficult to fit into one of the three classes. All thase cells are interspersed with common neurons which again vary in size.

The NSC of this locus are more abundant in anterior sections of the brain and posteriorly decrease in number progressively up to the appearance of locus 4 NSC.

Some of the 3b and 3c NSC lie close to the anterior border of the protocerebral neuropile (Fig. 85) whilst others lie close to hte median fissure (Figs 83, 85, 86, 87 and 88). Two or three class 3b and 3c pyriform NSC occur just below, and a few lie lateral to, the point where the median fissure meets the protocerebral neuropile (Figs 86, 87). The axons of these cells run antero-laterally for a short distance and then presumably turn posteriorly to join the NCCI before it crosses over to the other half of the protocerebrum. The axons of all the other NSC of locus 3 run vertically

Figure 87. Photomicrograph of a frontal section of protocerebrum of an adult ♀ P. americana, killed 48 hours after moulting, showing two medium-sized neurosecretory cells of locus 3 lying just below the point where the median fissue (MF) meets the protocere-:bral neuropile (PRCNp). The axons of these cells run antero-laterally while those of the other cells run vertically downwards. LGC, lateral globuli cells; 3a NSC, large neurosecretory cell of locus 3; 3b NSC, medium-sized neurosecre-:tory cells of locus 3; 3c NSC, small neurosecretory cells of locus 3. Note also sparse PF^+ neurosecretory granules (Gr) in the neurosecretory cells.

Figure 88. Photomicrograph of a frontal section of protocerebrum of a freshly moulted nymph of P. americana, showing the neurosecretory cells of locus 2 and 3. 2 NSC, neurosecretory cells of locus 2; 3 NSC, neurosecretory cells of locus 3. Other lettering as in Figure 87.

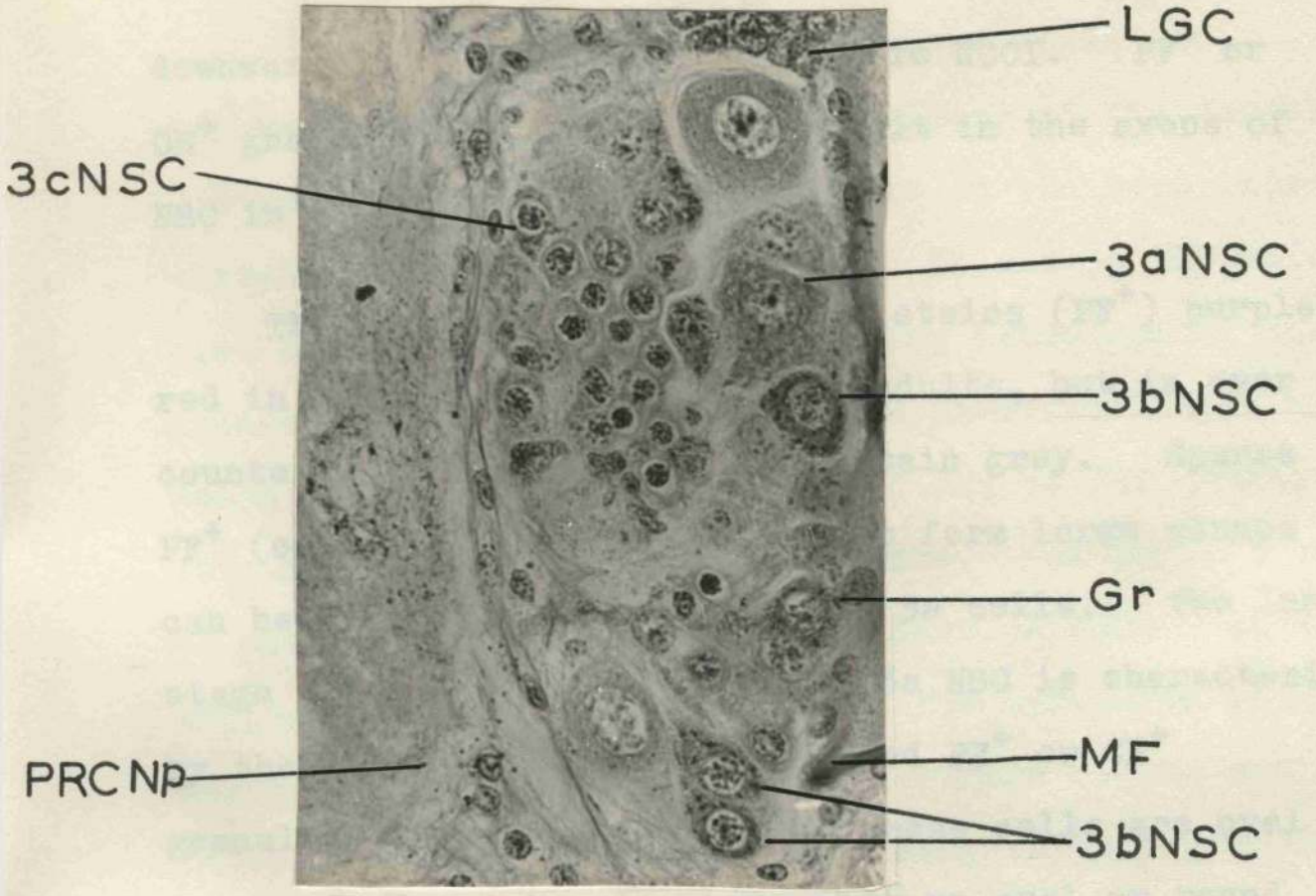


Figure 87.

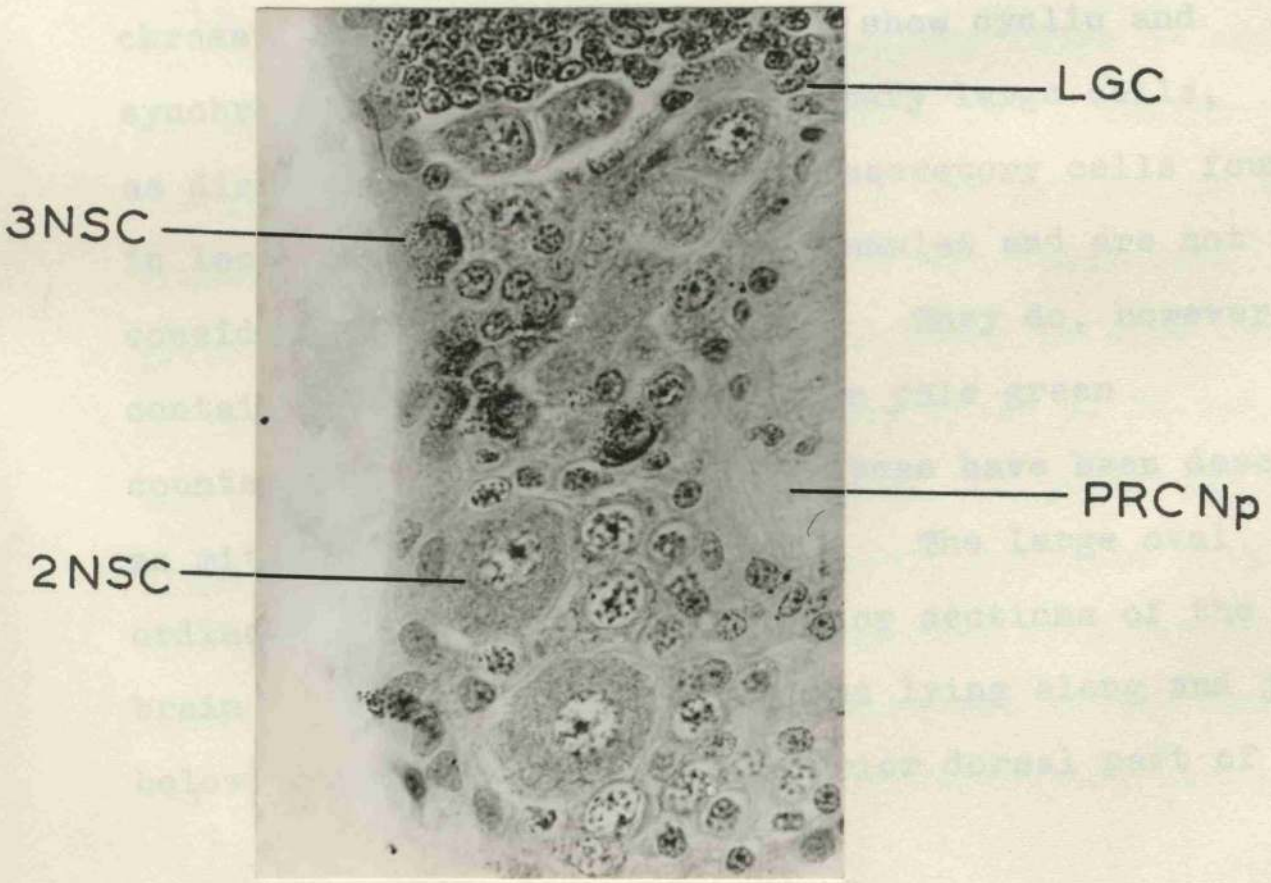


Figure 88.

downwards to join the prechiasmatic NCCI. PF^+ or CH^+ granules can be seen in transit in the axons of NSC in this location.

The cytoplasm of the 3a NSC stains (PF^+) purple-red in newly moulted nymphs and adults, but in over counterstained sections it may stain grey. Sparse PF^+ (or CH^+) granules which do not form large clumps can be seen in the perikaryon of 3a cells. The last stage in the secretory cycle of 3a NSC is characterized by the presence of a few scattered PF^+ or CH^+ granules and a few vacuoles. These cells are oval or round or angular in shape with an oval or round nucleus containing a distinct nucleolus and some chromatin material. The 3a NSC show cyclic and synchronous activity. The ordinary large cells, as distinct from the large neurosecretory cells found in locus 3 have no PF^+ or CH^+ granules and are not considered to be neurosecretory. They do, however, contain inclusions which take the pale green counterstain indigo carmine. These have been described as mitochondria by Pipa (1961b). The large oval ordinary neurons occur in anterior sections of the brain more or less like a garland lying along and just below the perilemma of the anterior dorsal part of the

protocerebrum. These ordinary nerve cells are, however, not confined to the periphery of the dorsal groove but occur in several layers in the pars inter-:cerebralis intermingled with the 3b and 3c NSC which also occur in several layers. The cytoplasm of the non-secretory neurons of the pars intercerebralis which compare in shape and size with 3b and 3c NSC counter-:stains grey after the PF procedure. In locus 3 the 3b and 3c cells outnumber the larger 3a NSC. At the beginning of the secretory cycle the cytoplasm of 3b and 3c NSC stains a uniform pink-purple colour and does not contain PF^+ granules, this being the non-granular phase in the secretory cycle of these cells. Later a few light purple granules appear. These gradually increase in number and form aggregates (Figs 84 and 89) which stain deep purple. These PF^+ granules ultimately fill the whole space in the perikaryon and axon-hillock. As accumulation of neurosecretory granules proceeds, these cells increase in size but in the last stage of the secretory cycle of 3b and 3c NSC there is a reduction in size of the cells and the cytoplasm, now grey-green in colour, contains only a few PF^+ granules which are accompanied by a few small vacuoles. In newly moulted nymphs and adults some of the 3b and 3c NSC are full of neurosecretory granules

Figure 89. Photomicrograph of a medium-sized (3b NSC) and a small (3c NSC) neurosecretory cell of locus 3 showing abundant aggregated PF^+ granules in perikaryon and axon-hillock. Adult P. americana killed within 1 hour after the moult.

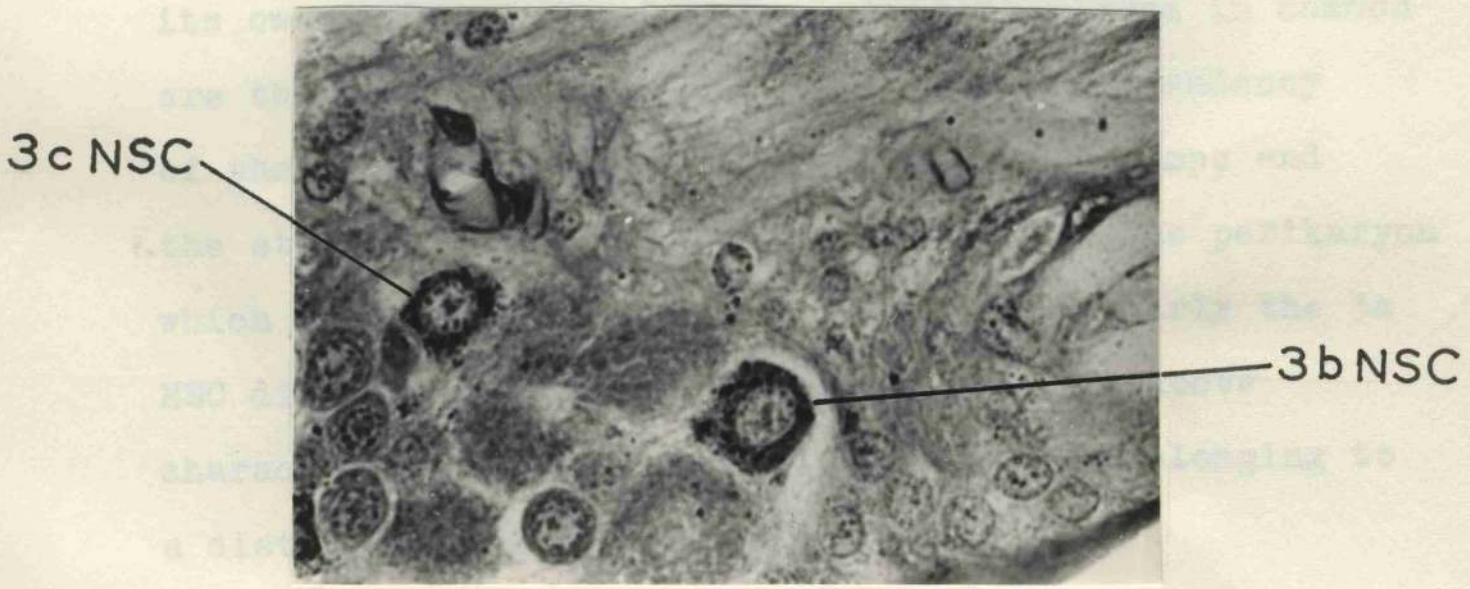


Figure 89.

but on the whole this secretory activity is cyclical but asynchronous. It may be that each 3b and 3c NSC has its own secretory cycle. At a given time there is more neurosecretory material present in 3b and 3c NSC than in 3a NSC. 3b and 3c NSC are not considered to represent stages in the neurosecretory cycle of the same class of cell but different classes, each with its own cycle. The features which they have in common are their shape, asychronous activity, the tendency of their neurosecretory granules to form clumps and the storage of neurosecretory material in the perikaryon which makes these cells so prominent. Clearly the 3a NSC differ from the 3b and 3c cells in the above characteristics and are to be regarded as belonging to a distinct class.

Locus 4

Locus 4 NSC can be seen when the calices and their peduncles have fully appeared in frontal sections. Locus 4 consists of the following NSC in each half of the protocerebrum: one giant neuron (4a) (about 60-80μ) which is round or oval in section and lies ventro-lateral to the internal calyx (Fig. 90). This is the large cell described as neurosecretory by Drescher (1960). A thick axon arises from this neuron.

Figure 90. Photomicrograph of a frontal section of protocerebrum of an adult ♂ P. americana, killed 1 hour after moulting, showing two giant neurons of locus 4 (4a NSC) with fine PF^+ granules (Gr) in the perikaryon and axon-hillock. ML, median line dividing the brain into two equal halves; IntCX, internal calyx; LGC, lateral globuli cells; N, ordinary neurons lying ventral to the large cells.

Figure 91. Photomicrograph of a frontal section of protocerebrum of a freshly moulted nymph of B. orientalis showing a large neuron (4b) with fine blue-black (CH^+) granules (Gr). Lettering as in Figure 90.

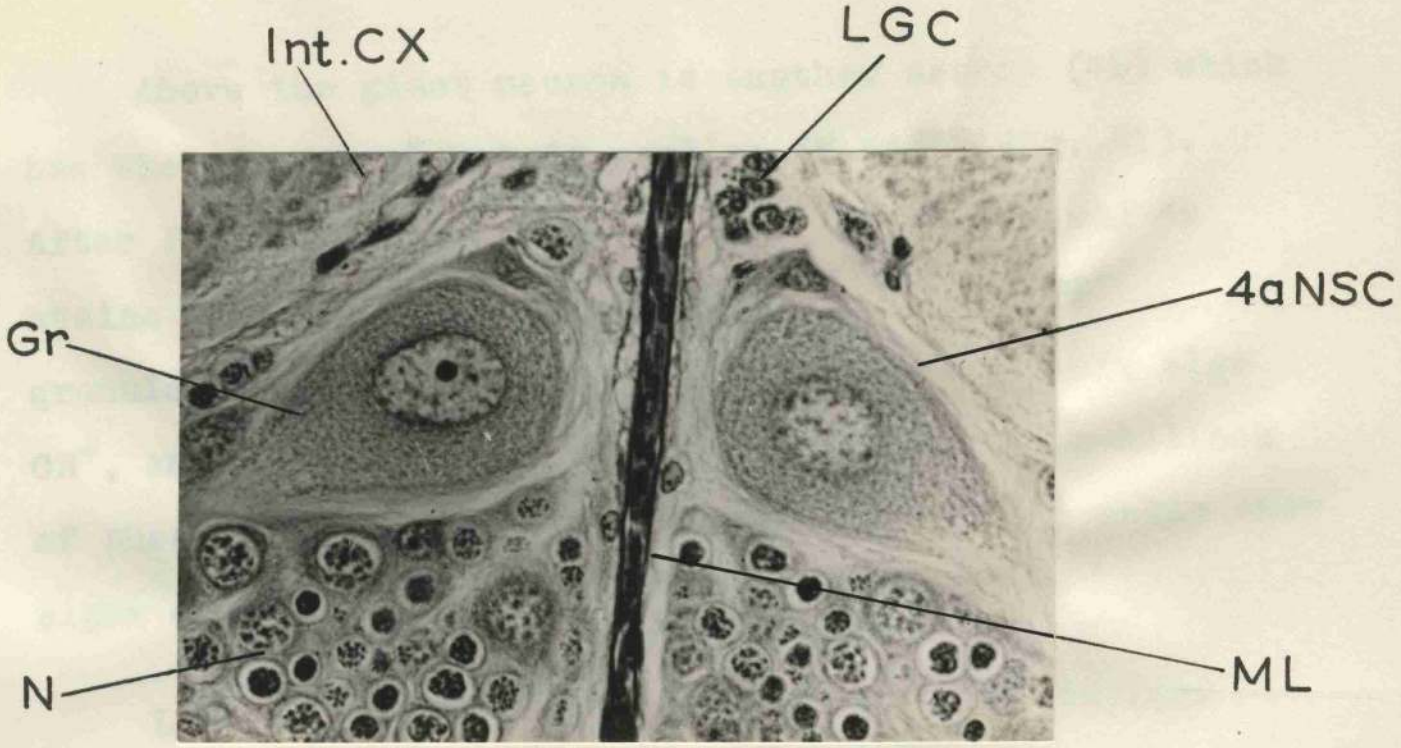


Figure 90.

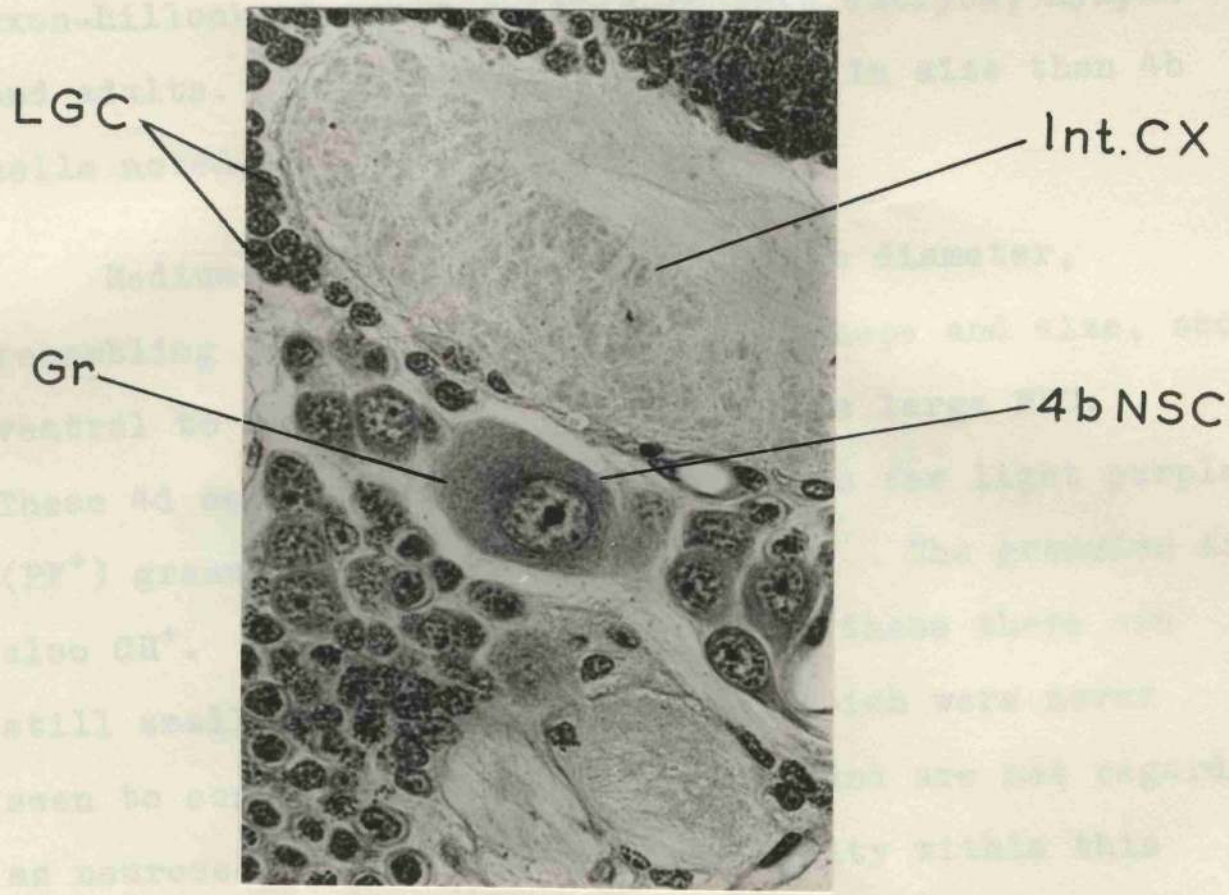


Figure 91.

Above the giant neuron is another neuron (4b) which has the same shape but is smaller in size (Fig. 91). After PF staining the cytoplasm of neurons 4a and 4b stains pink or sometimes green and possesses PF^+ granules and small vacuoles. These granules are also CH^+, and can be seen in the perikaryon and axon-hillock of these cells but not in their axons. These cells show signs of cyclical secretory activity.

Lying ventro-lateral and ventral to the calices are other large cells (4c) some of which (Fig. 92) possess PF^+ or CH^+ granules in their perikaryon and axon-hillock at certain times in late embryos, nymphs and adults. These cells are smaller in size than 4b cells noted above.

Medium-sized cells (4d) 20-25μ in diameter, resembling the 3b NSC of locus 3 in shape and size, occur ventral to the calices and also to the large NSC. These 4d cells (Figs 92, 93) possess a few light purple (PF^+) granules and reddish cytoplasm. The granules are also CH^+. Ventral and posterior to these there are still smaller nerve cells (10-15μ) which were never seen to contain PF^+ or CH^+ granules and are not regarded as neurosecretory. Secretory activity within this locus is synchronous and in phase with activity in loci

Figure 92. Photomicrograph of a section of protocere-
:brum of an adult ♀ P. americana, killed
18 hours after the moult, showing a large
cell (4c) of locus 4 having a few fine
purple (PF^+) granules. The cell lies
ventral to the internal calyx (IntCX).
Note also a medium-sized (4d) cell which
also possesses sparse fine PF^+ granules.
This cell is lying ventro-lateral to the
internal calyx.

Figure 93. Photomicrograph of a section of protocere-
:brum of an adult ♀ P. americana, killed
18 hours after the moult, showing a medium-
sized cell (4d) of locus 4. The cell has
fine PF^+ granules. The cell lies ventral
to the internal calyx. Note also the
small ordinary neurons lying ventral to
4d cells.

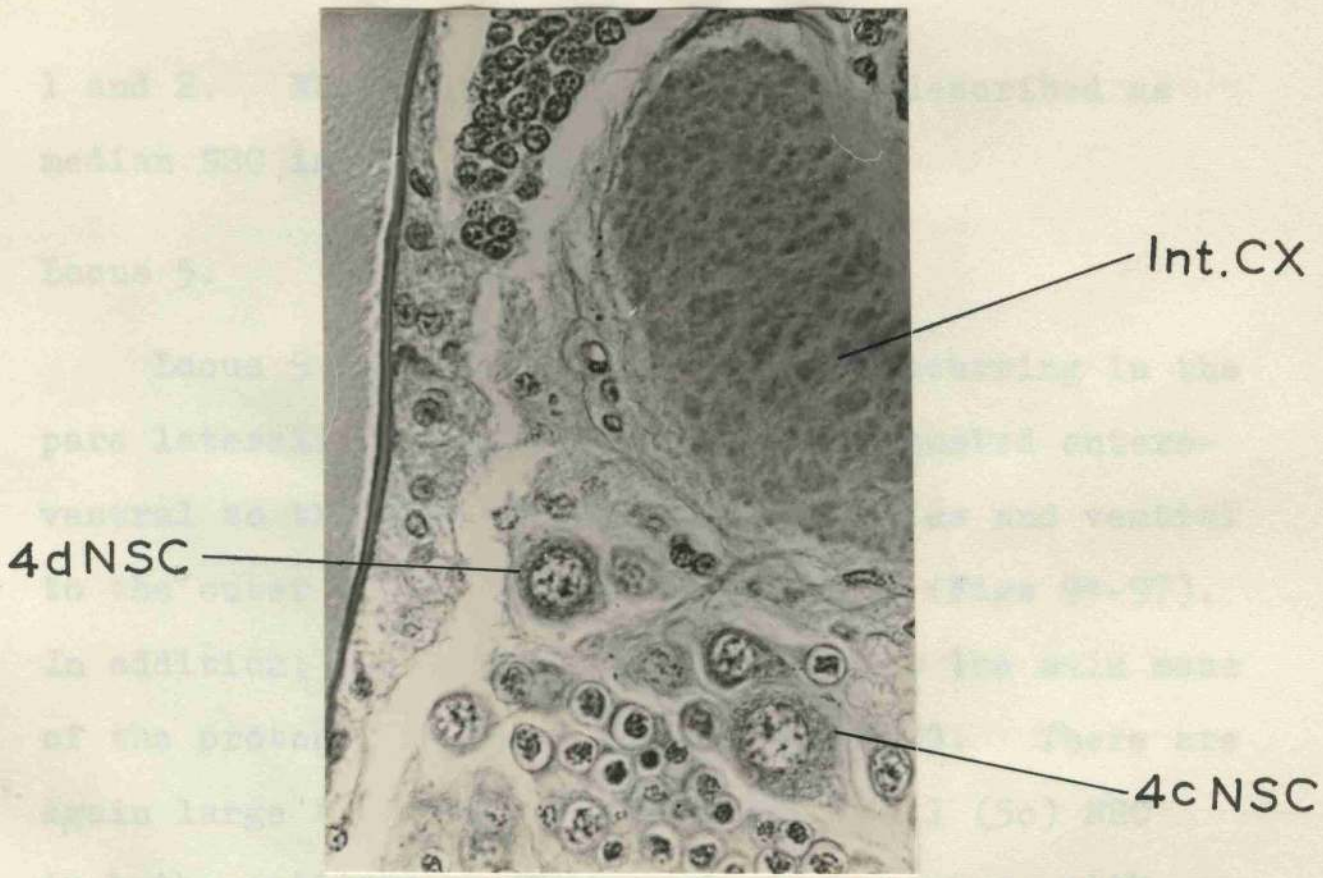


Figure 92.

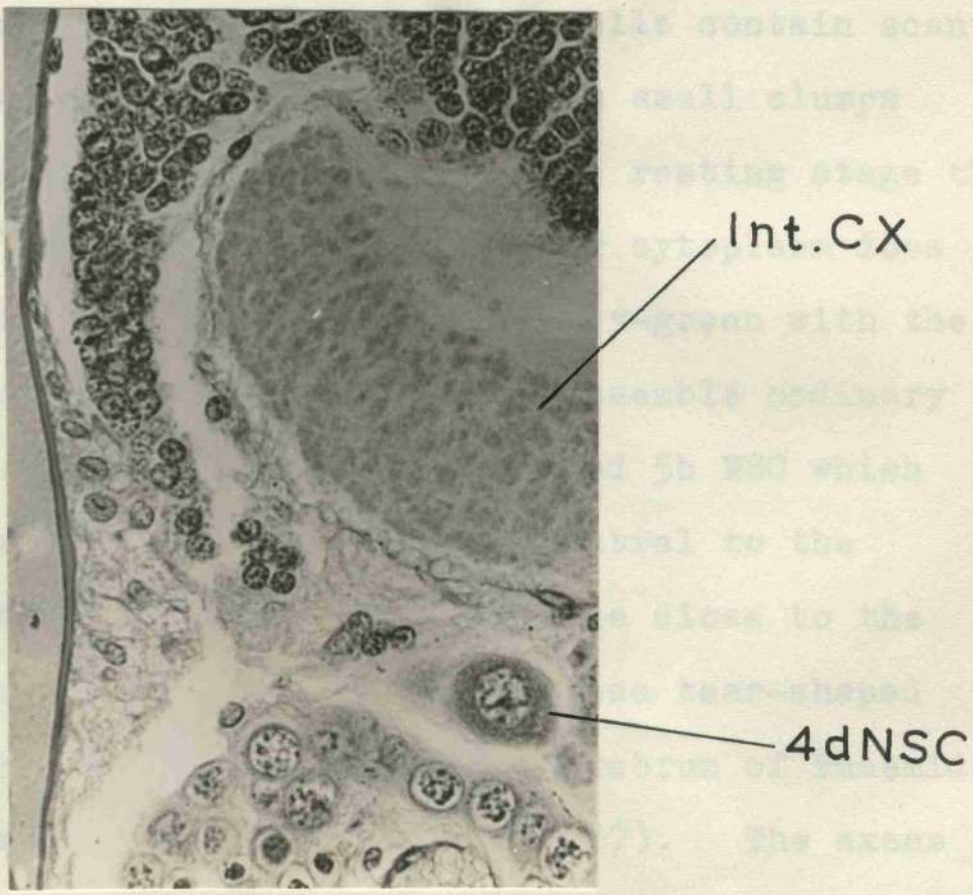


Figure 93.

1 and 2. NSC of loci 2, 3 and 4 are described as median NSC in Chapter 3.

Locus 5.

Locus 5 consists of lateral NSC occurring in the pars lateralis of the protocerebrum situated antero-ventral to the free end of the cauliculus and ventral to the outer end of the external calyx (Figs 94-97). In addition, other NSC occur lateral to the main mass of the protocerebral neuropile (Fig. 98). There are again large (5a), medium (5b), and small (5c) NSC in both positions. Their cytoplasm stains, with paraldehyde fuchsin, uniformly pink or purple especially in newly moulted specimens. These cells contain scanty PF^+ or CH^+ granules some of which form small clumps while the rest are scattered. In the resting stage these NSC lack PF^+ or CH^+ granules and their cytoplasm does not stain with PF though it stains grey-green with the counterstains. At this time these resemble ordinary nerve cells. Some of the medium-sized 5b NSC which are tear-shaped (Fig. 97) lie just ventral to the lateral globuli cells which in turn lie close to the outer end of the external calyx. These tear-shaped cells resemble the NSC in the tritocerebrum of Phasmids photomicrographed by Dupont-Raabe (1957). The axons

Figure 94. Photomicrograph of a large (5a) and two medium-sized (5b) neurosecretory cells lying antero-ventral to the free end of the cauliculus (CAU). The cells have fine PF^+ granules. Adult ♀ P. americana killed 18 hours after moulting.

Figure 95. Photomicrograph of a medium-sized (5b) neurosecretory cell lying ventral to the free end of the cauliculus (CAU). The cell has fine scattered PF^+ granules. Last instar ♀ nymph of P. americana killed 90 days after moulting.

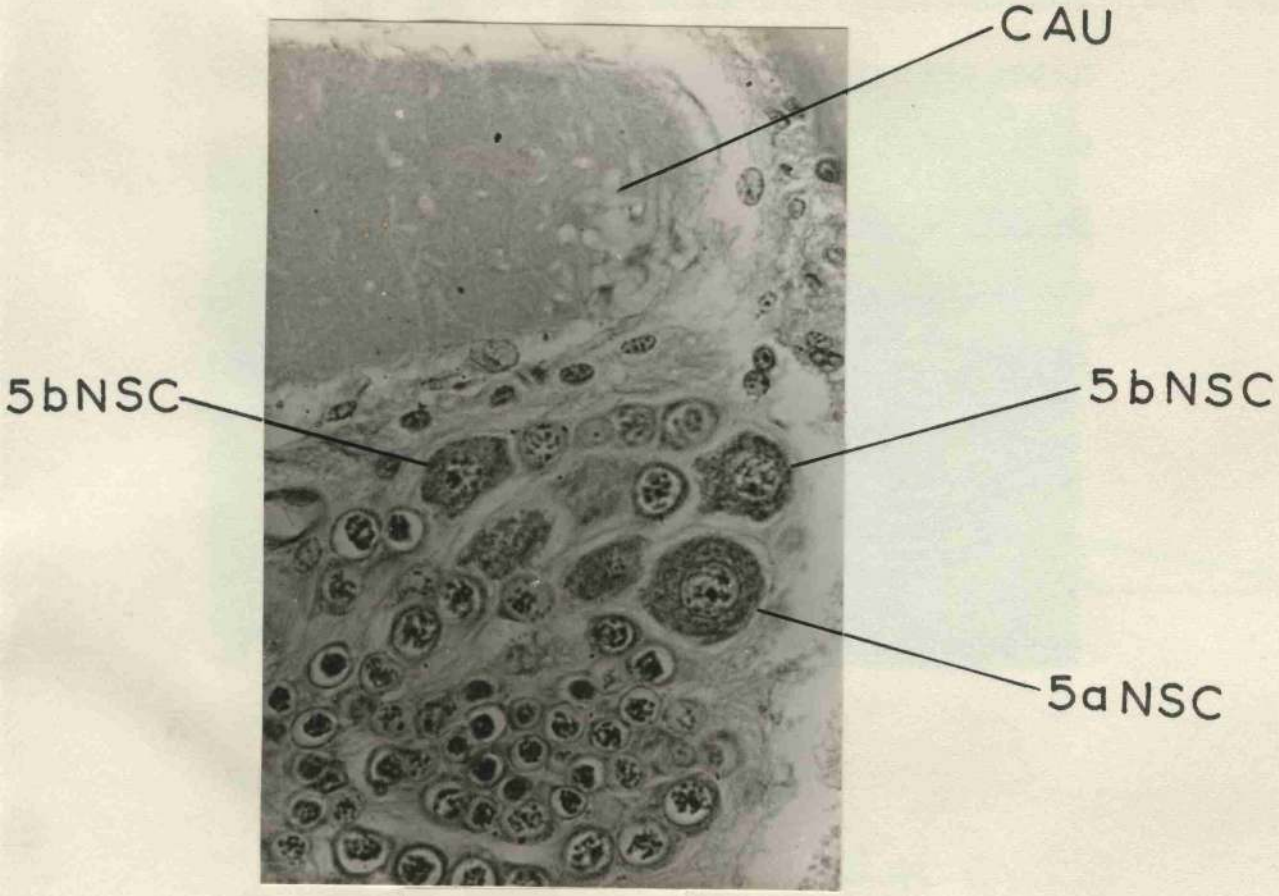


Figure 94.

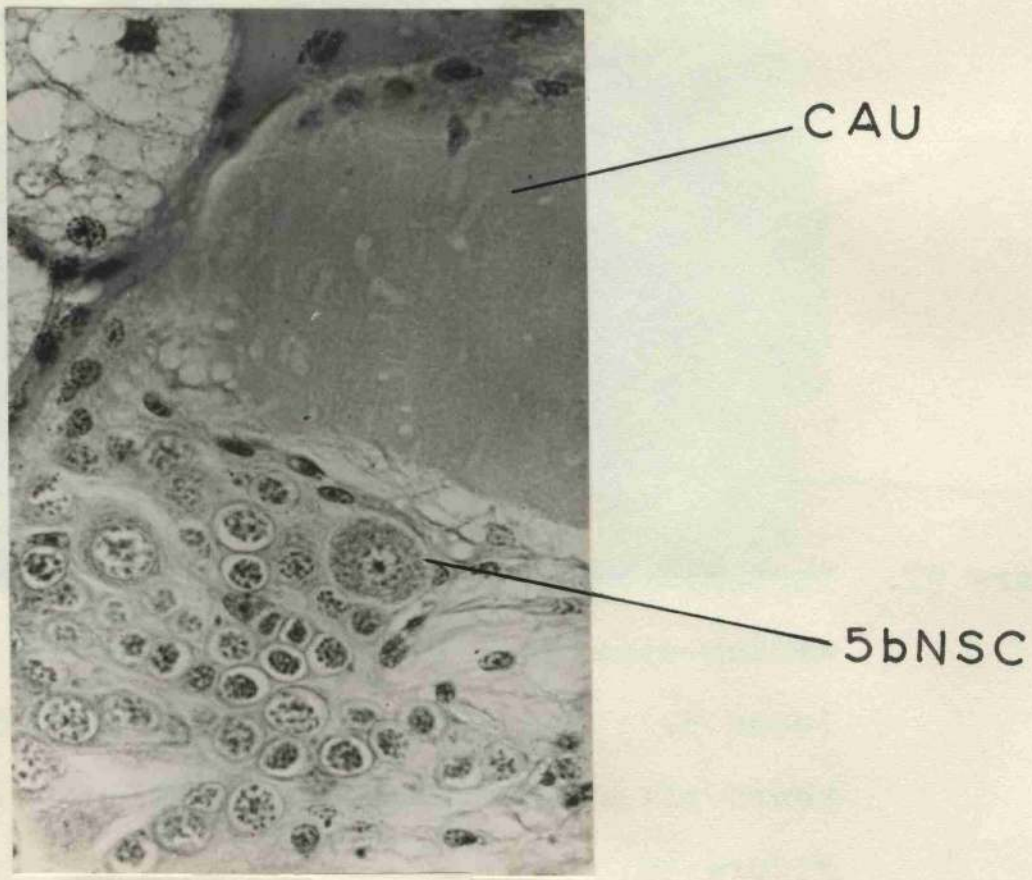


Figure 95.

Figure 96. Photomicrograph showing two small (5c) neurosecretory cells of locus 5. The cells have PF^+ granules. Adult ♀ P. americana killed 18 hours after moulting. Ext.C, external calyx; LGC, lateral globuli cells; CAU, cauliculus.

Figure 97. Photomicrograph showing a tear-shaped medium-sized (5b) neurosecretory cell of locus 5. Adult ♀ P. americana killed 18 hours after moulting. Lettering as in Figure 96.

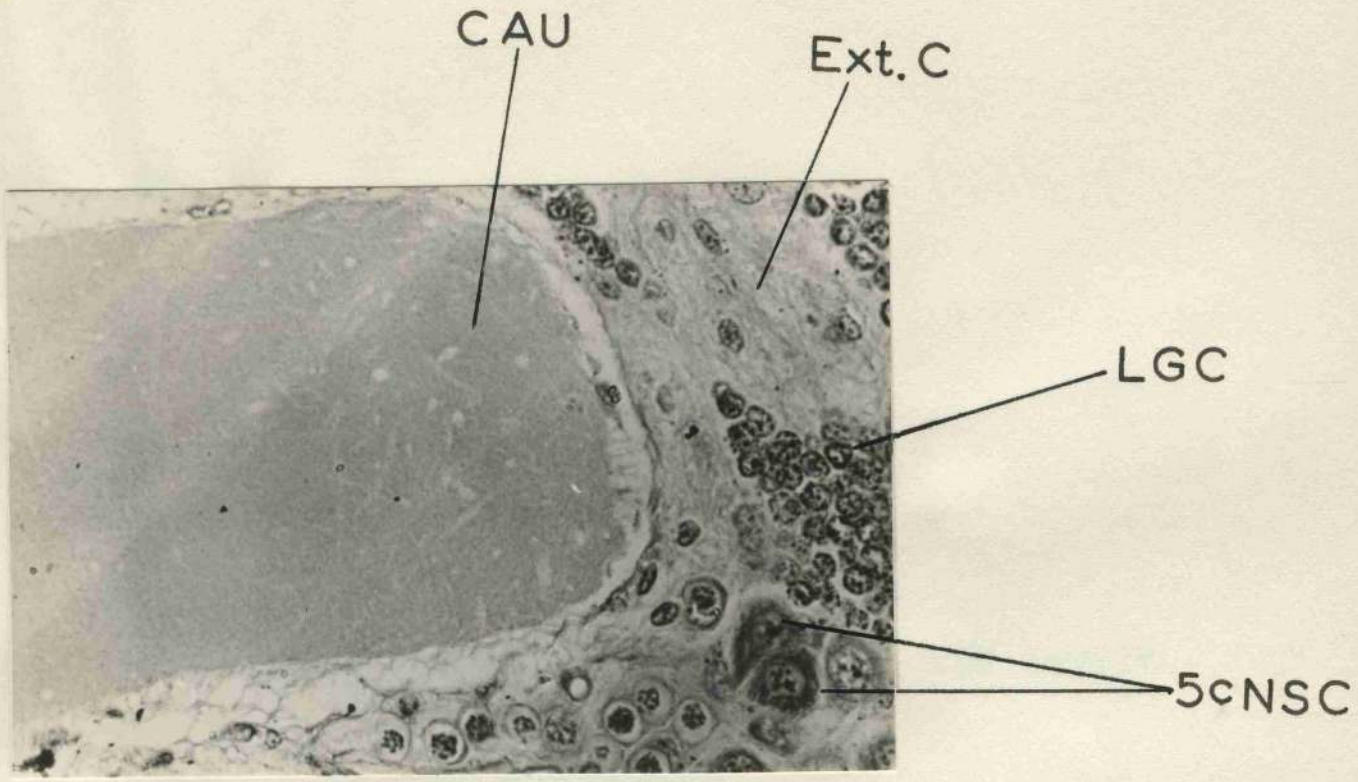


Figure 96.

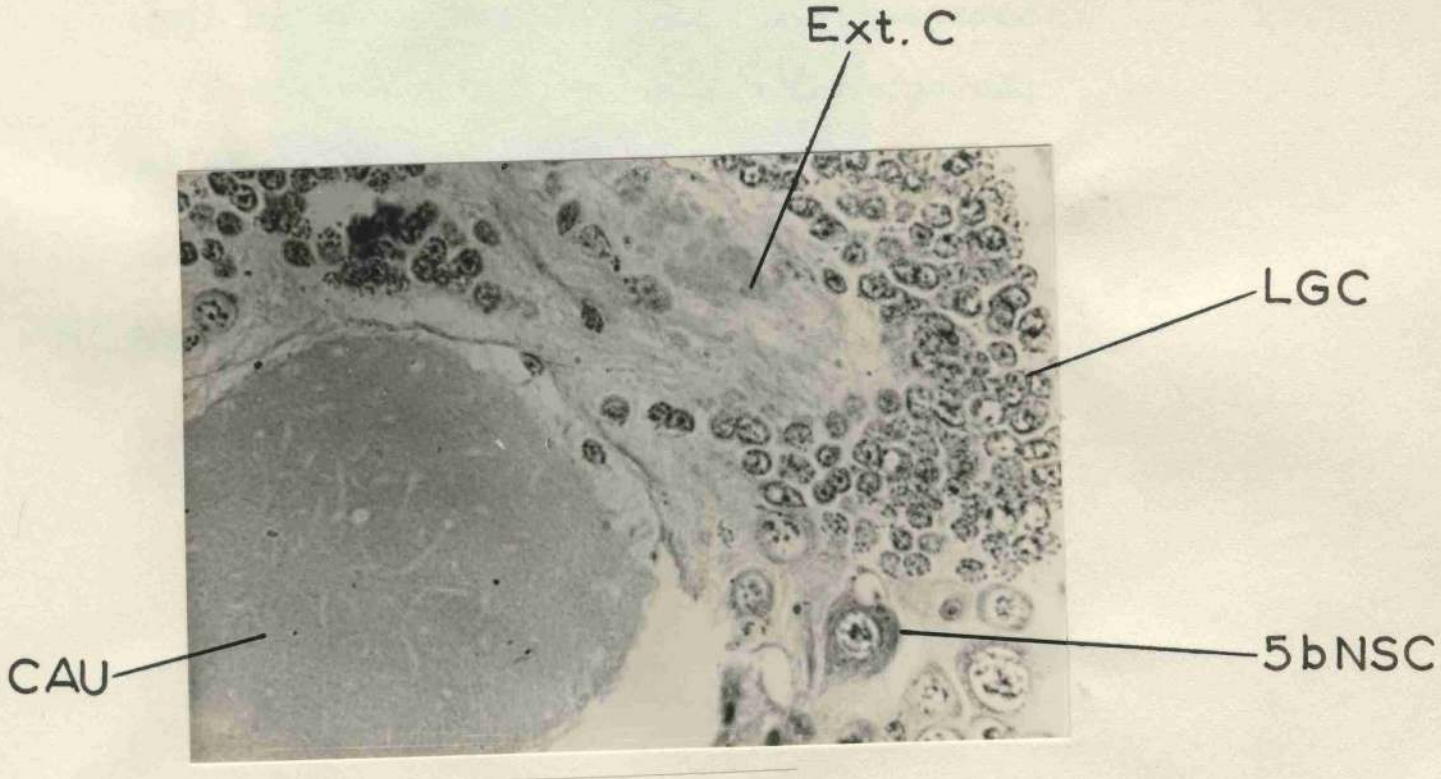


Figure 97.

Figure 98. Photomicrograph showing a medium-sized neurosecretory cell (NSC) lying lateral to the main mass of the protocerebral neuropile (PRCNp). Note also a large neurosecretory cell (shown above in the photograph) which lies antero-ventral to the cauliculus. Both of these cells have fine PF^{+} granules. Adult ♂ P. americana killed 1 hour after moulting.

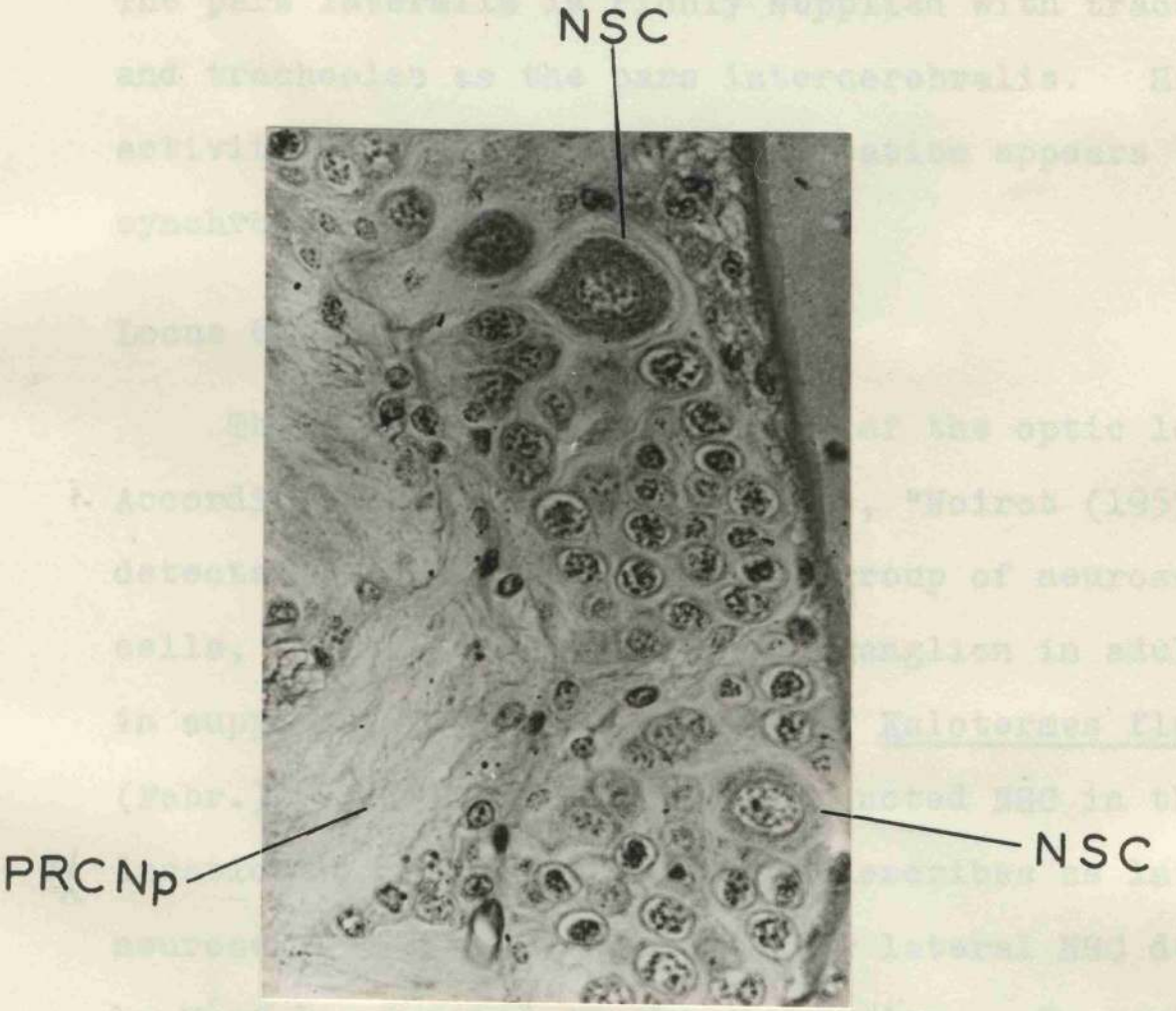


Figure 98.

of these NSC form the NCCII (see Chapter 3). Willey (1961) describes in P. americana the lateral NSC, which he includes in his group 5, but he says "I have not investigated the neurosecretion of this area thoroughly". The pars lateralis is richly supplied with tracheae and tracheoles as the pars intercerebralis. Secretory activity in the cells of this location appears to be synchronous.

Locus 6.

This locus contains the NSC of the optic lobe. According to Van der Kloot (1960), "Noirot (1957) detects secretory activity in a group of neurosecretory cells, at the base of the optic ganglion in adult and in supplementary reproductives of Kalotermes flavicollis (Fabr.)". Köpf (1957) has also noted NSC in this location in Drosophila which he describes as lateral neurosecretory cells. The other lateral NSC described by Kopf lie ventral to the trabeculae. In newly moulted nymphs and adults of the cockroaches studied there are two or three large cells (6a) (Fig. 99) lying just ventral to the medulla interna of the optic lobe. These large cells and a medium-sized cell (6b) and a smaller cell (6c) (Fig. 100) lying anterior to them, show, with CHP staining, fine scattered CH^+ granules

Figure 99. Photomicrograph showing a large cell (6a) of locus 6 having PF^+ granules. Adult ♀ P. americana killed just after the moult.

Figure 100. Photomicrograph showing a medium-sized (6b) and a small cell (6c) of locus 6 with a few PF^+ granules. These cells lie anterior to the large cell shown in Figure 99. Adult ♀ P. americana killed just after the moult.

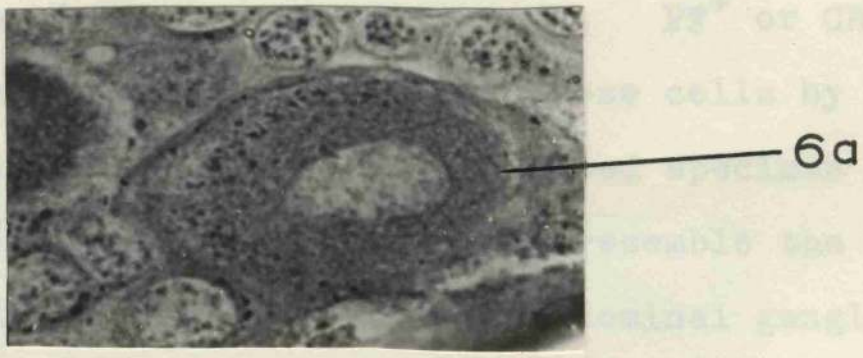


Figure 99.

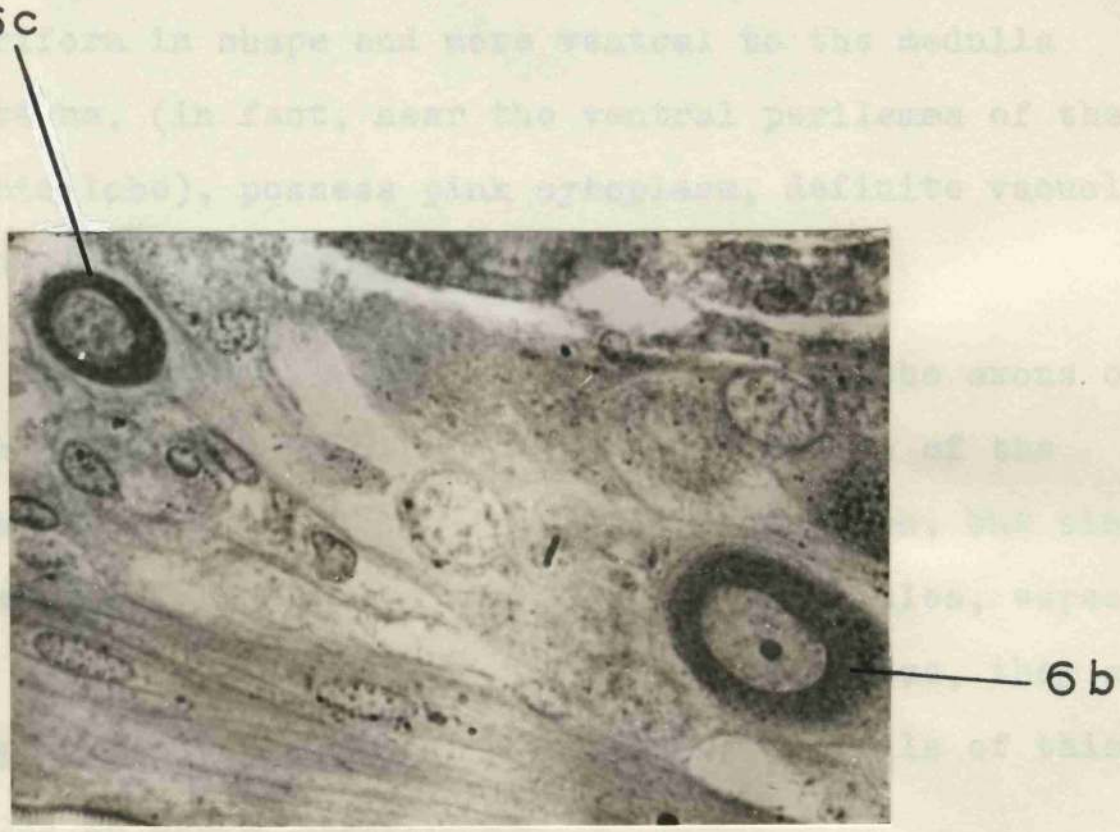


Figure 100.

and purplish cytoplasm and, with PF staining, pinkish cytoplasm and scattered but distinct PF^+ granules. These cells appear oval or pyriform in section with an oval nucleus which has a distinct nucleolus and may possess abundant chromatin material. PF^+ or CH^+ granules are usually absent from these cells by the time the cuticle of a recently moulted specimen has hardened and darkened. These NSC resemble the type A cells of the thoracic and abdominal ganglia of P. americana and B. orientalis photomicrographed by Füller (1960) in his figures 9a-c. In addition, a few cells of medium size (6d) (Fig. 101) more or less pyriform in shape and more ventral to the medulla interna, (in fact, near the ventral perilemma of the optic lobe), possess pink cytoplasm, definite vacuoles, and a few PF^+ granules.

PF^+ or CH^+ granules were not seen in the axons of the NSC of this locus, and the destination of the granules produced by the cells is not known, but since these nerve cells possess CH^+ or PF^+ granules, especially in newly moulted cockroaches, as noted above, they are regarded as NSC. The activity of the cells of this locus is synchronous.

Figure 101. Photomicrograph showing a medium-sized (6d) cell of locus 6. The cell has a few PF^+ granules and vacuoles. Adult ♀ P. americana killed just after the moult.

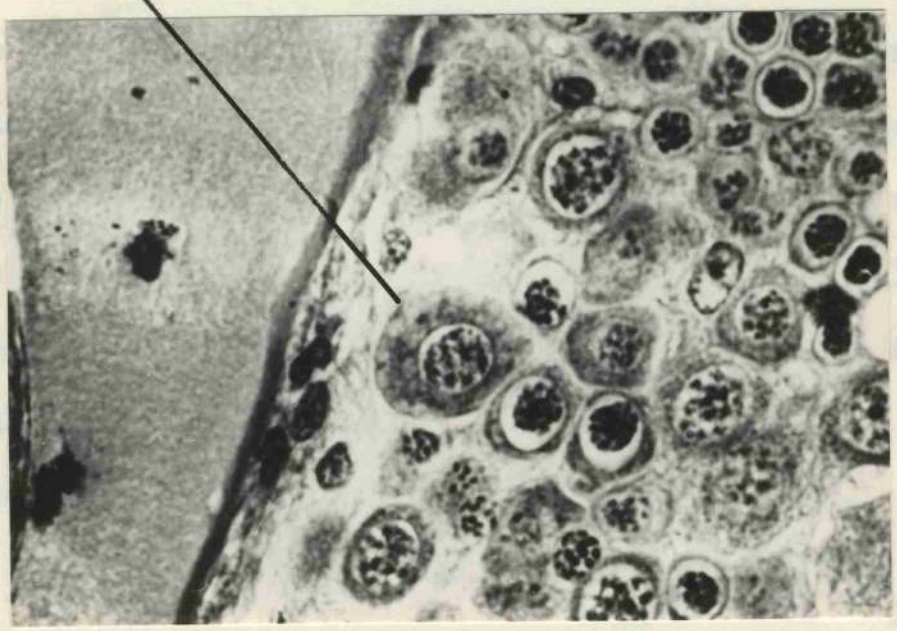


Figure 101.

Ib NSC in the deutocerebrum

Neurosecretory cells in the deutocerebrum have been described by Grandori (1955) in pupae of Calliphora and Musca, and by Noirot (1957) in Kalotermes. In the two species of cockroaches studied here cortical cells of different sizes were found, in the anterior part of the deutocerebrum, which show cytological signs of neurosecretion. Fine CH^+ or PF^+ granules form small or large aggregates which may or may not fill the whole perikaryon (Fig. 102). PF^+ granules were seen in large amounts in the axon-hillock and could be traced in the axon for a short distance. Sometimes the PF^+ granules do not form clumps and are confined only to the abaxonal region of the cell.

The activity of the cells appears to asynchronous as is indicated by the varying amount of PF^+ granules and the different arrangements of PF^+ granules in neighbouring cells. The destination of these granules is not known. Cortical cells were found in the olfactory lobe with diffuse purple cytoplasm and a few PF^+ granules. These were first seen in 42-day embryos and in all nymphal and adult stages. The granules were more abundant in newly moulted animals than after the moult; e.g. more PF^+ granules were found in the

Figure 102. Photomicrograph of a neurosecretory cell (NSC) in the deutocerebrum of an adult ♂ P. americana killed 1 hour after the moult. The cell has an appreciable amount of PF^+ granules.

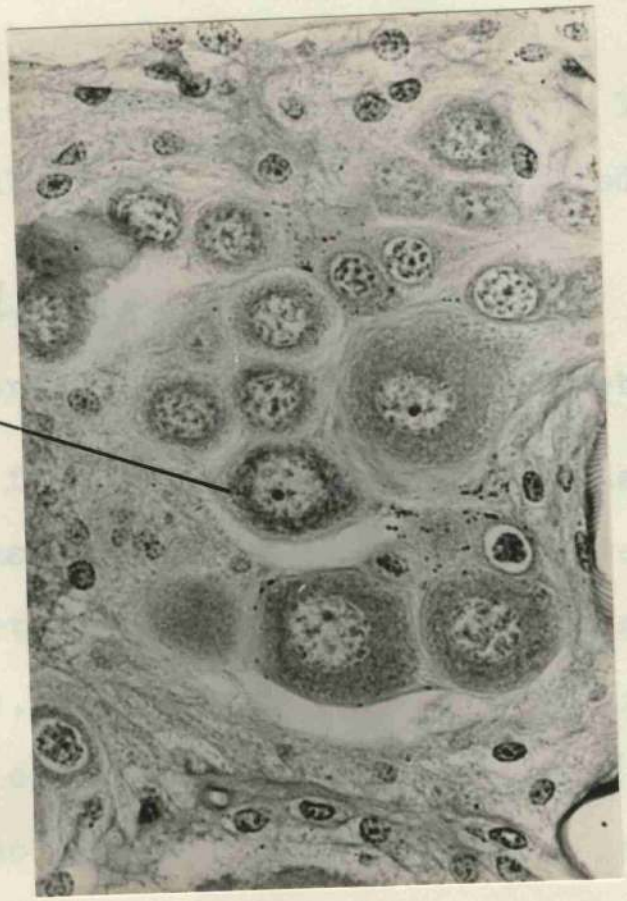


Figure 102.

anterior cortical cells in newly moulted adults than in adults 48 hours after moulting. In a 48-hour adult female and in older females, a few anterior cortical cells have scanty PF^+ granules in the perikaryon and axon-hillock and sometimes the granules actually appear to be lodged in vacuoles. There are more PF^+ granules in the cortical cells of later nymphal instars and adults than in late embryos and early nymphal instars.

Ic NSC in the tritocerebrum

(1) Cortical NSC in the tritocerebrum

A few medium-sized cortical nerve cells with PF^+ cytoplasm and a few PF^+ granules were observed in almost all parts of the tritocerebrum in some of the late embryos, first instar nymphs, later nymphal instars and adults of P. americana (Fig. 103). Neither the secretory cycle not the destination of the secretory material of the NSC of tritocerebrum has been worked out as yet. The destination of the neurosecretory material may well be the corpora cardiaca, via the NCCIII, but in the author's opinion the whole question of neurosecretion in the tritocerebrum in the cockroaches needs further study (see Chapter 3). Willey (personal communication) did not find NSC in the tritocerebrum of cockroaches though Dupont-Raabe (1957) reports $Azan^+$ NSC in the

Figure 103. Photomicrograph showing the tritocerebrum (TRC) and a cell (C) with a few PF^+ granules. Adult P. americana ♂ killed 1 hour after the moult.

Figure 104. Photomicrograph of a large cell in circum-oesophageal connectives close to their junction with the suboesophageal ganglion. The cell has PF^+ granules. Adult ♀ P. americana killed just after the moult.

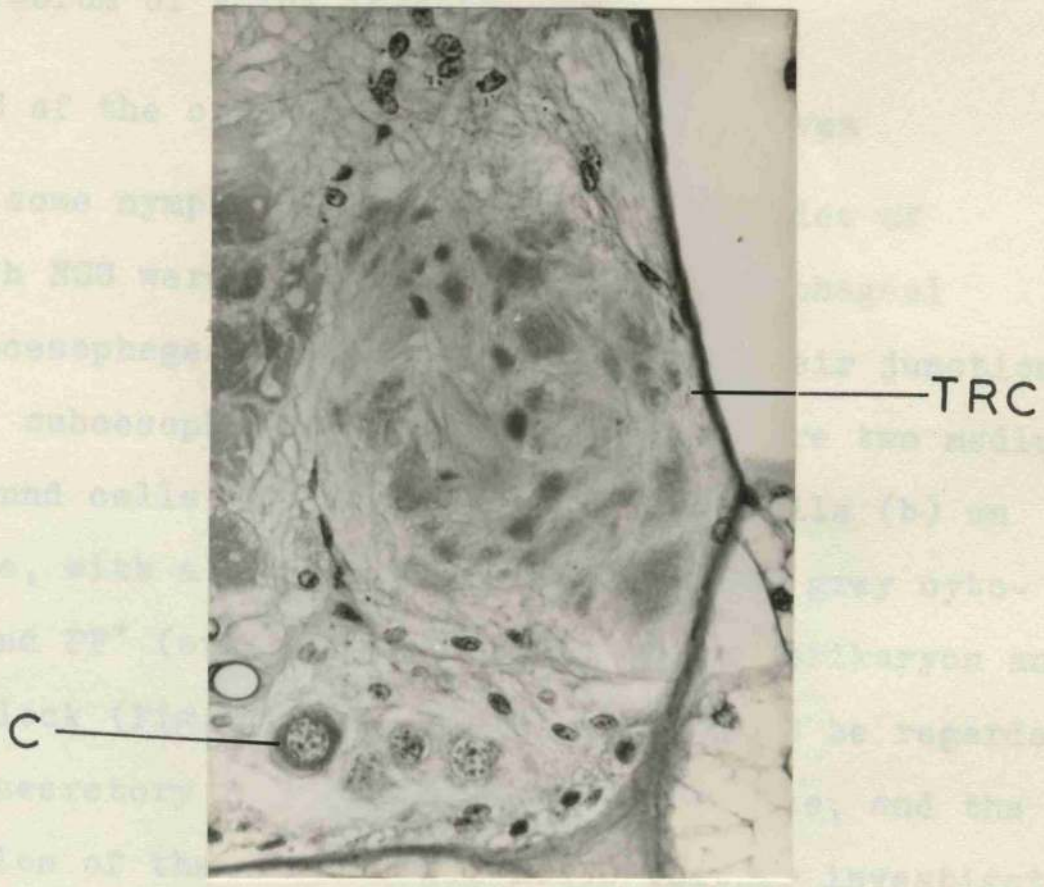


Figure 103.

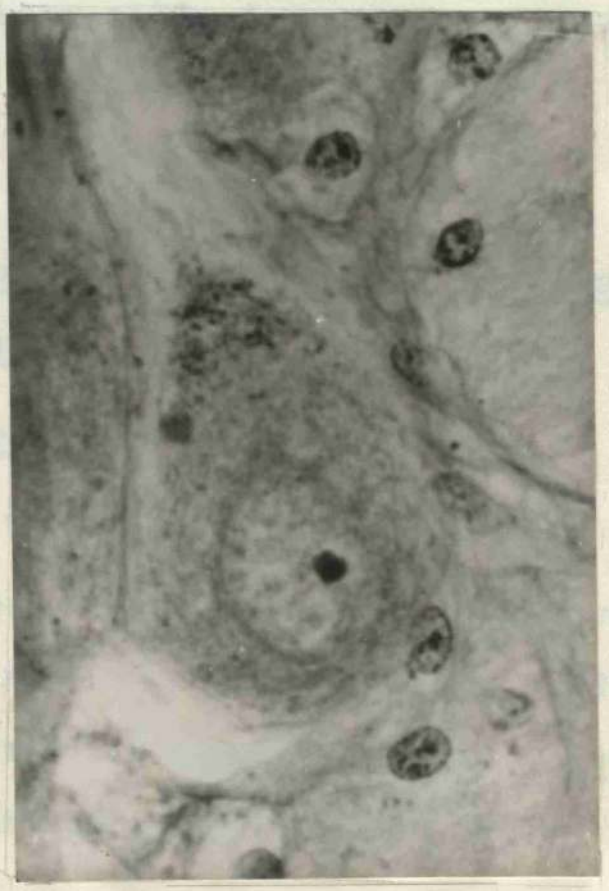


Figure 104.

tritocerebrum of stick insects.

(2) NSC of the circum-oesophageal connectives

In some nymphs and adults of both species of cockroach NSC were found in the circum-oesophageal or para-oesophageal connectives close to their junction with the suboesophageal ganglion. These are two medium-sized round cells (a) and two large oval cells (b) on each side, with a light purple or sometimes grey cyto-:plasm and PF^+ (or CH^+) granules in their perikaryon and axon-hillock (Fig. 104). These cells might be regarded as neurosecretory on the cytological evidence, and the destination of their secretion needs further investigation. However, the large cells mentioned above resemble the type 1 neurosecretory cells described and figured by Geldiay (1959) in the thoracic and abdominal ganglia of Blaberus.

Section II: Neurosecretory Cells in Other Parts of the Central Nervous System

IIa NSC in the frontal ganglion

According to Willey (1961) the cells of the frontal ganglion of cockroaches do not stain selectively with paraldehyde fuchsin and according to Dupont-Raabe (1958) the cells of the frontal ganglion of Carausius do not stain with any neurosecretory stain. However, in the

Figure 105. Photomicrograph showing nerve cells of frontal ganglion. The cells have PF^+ granules. N, nerve cells; G, glia cells. Last instar ♀ nymph of P. americana killed 1 hour after moulting.

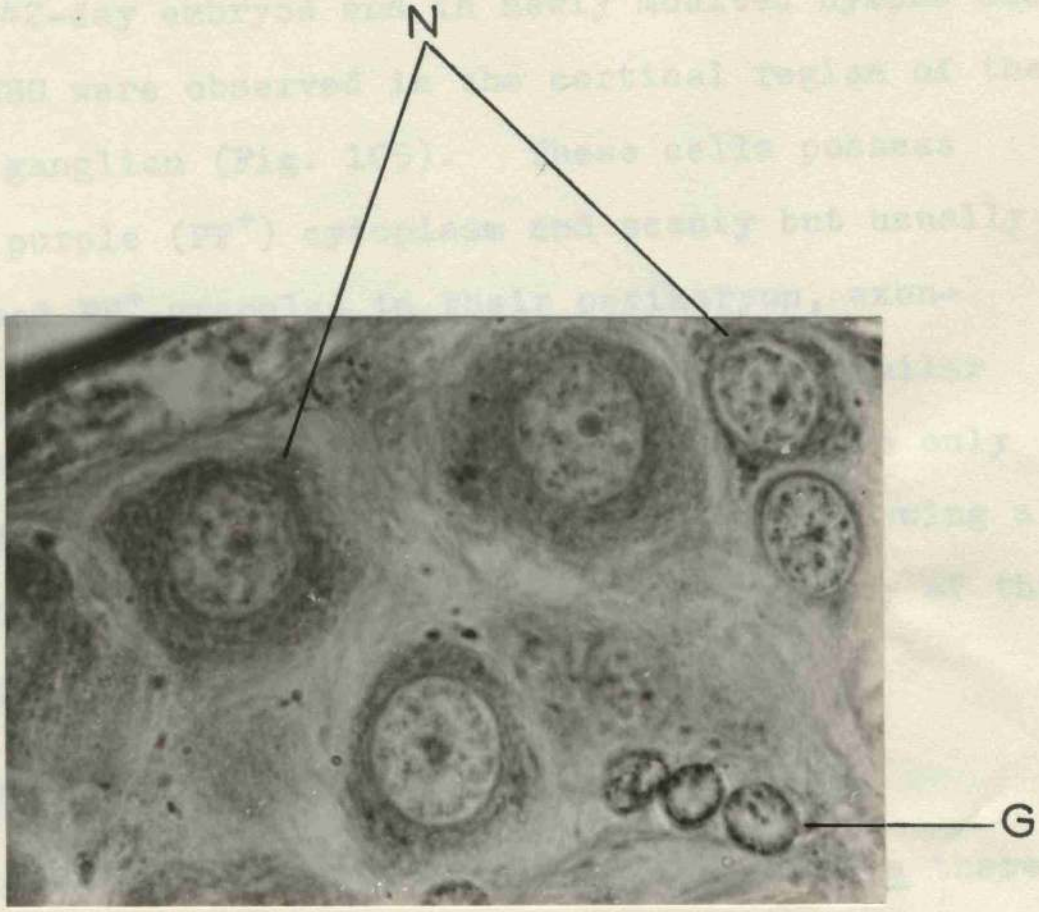


Figure 105.

review by Van der Kloot (1960) neurosecretory cells are said to occur in the frontal ganglion.

In 42-day embryos and in newly moulted nymphs and adults NSC were observed in the cortical region of the frontal ganglion (Fig. 105). These cells possess diffuse purple (PF^+) cytoplasm and scanty but usually aggregated PF^+ granules in their perikaryon, axon-hillock and axons. In tanned cockroaches, similar cells have counterstained cytpolasm and possess only a few PF^+ granules. The number of neurons showing a PF^+ reaction is indeed very low. The activity of the cells appears to be asynchronous.

IIb NSC in the suboesophageal ganglion

According to Füller (1960) in P. americana there are only three groups of neurosecretory cells in the SOG, lateral, ventro-medial, and ventro-lateral. But according to the present observations five groups of NSC are identifiable in the SOG of both P. americana and B. orientalis. These groups are:

1) antero-median,
2) antero-lateral,
3) latero-lateral,
4) ventro-lateral and

Figure 106. Diagram of the distribution of neurosecretory cells in the suboesophageal ganglion of a third instar nymph of P. americana, ready to moult within a few days. Ant.MNSC, antero-median neurosecretory cells; Ant. LNSC, antero-lateral neurosecretory cells; LLNSC, latero-lateral neurosecretory cells; VMNSC, ventro-median neurosecretory cells; VLNSC, ventro-lateral neurosecretory cell; LbN, labial nerve; NSGr, neurosecretory granules (PF^+); NP, neuropile of the suboesophageal ganglion; LLNSC(N), latero-lateral neurosecretory cell of a last instar ♂ nymph of P. americana, killed 89 days after moulting; LLNSC(A), latero-lateral neurosecretory cell of an adult ♀ P. americana, killed 18 hours after moulting; VMNSC(A), ventro-median neurosecretory cell of an adult ♀ P. americana, killed 18 hours after moulting, containing abundant fine PF^+ neurosecretory granules; VLNSC(A), ventro-lateral neurosecretory cell of an adult P. americana, killed 18 hours after moulting.

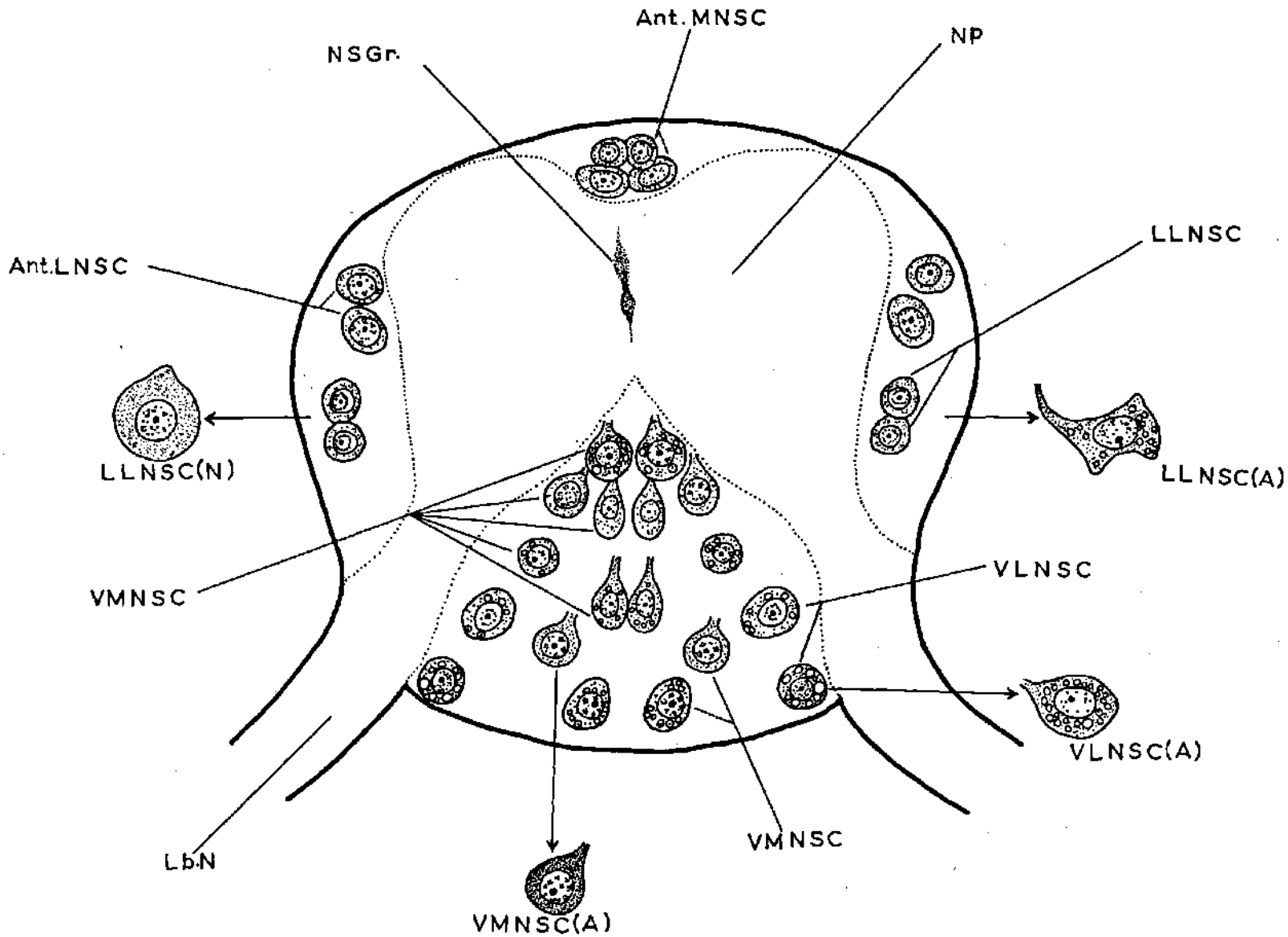

Ant.MNSC
NSGr.
NP
Ant.LNSC
LLNSC
LLNSC(N)
LLNSC(A)
VMNSC
VLNSC
VLNSC(A)
Lb.N
VMNSC
VMNSC(A)
25μ

Figure 106.

5) ventro-median (Fig. 106).

The majority of NSC in the SOG belong to the ventro-median group. Within this group the amount of PF^+ material in different cells is very variable. Füller (1960) states that development and storage of secretory products are similar in all the NSC of the SOG of P. americana and thus he could not separate the cells into types A and B as Scharrer (1955b) has done in the SOG of Leucophaea. He adds that it is a 'remarkable fact' that the NSC of the SOG do not possess 'intensive secretory products' as found in the NSC of the pars intercerebralis. The author must disagree with him entirely on these points since at least three classes of NSC large, medium and small, have been demonstrated in the present work in the SOG of both P. americana and B. orientalis. The medium and large NSC do not usually have as many neurosecretory granules as one particular pair of small ventro-median NSC, though the intensity of purple staining of the cytoplasm and the content of neurosecretory granules in these small cells vary depending on the stage in the secretory cycle at which they have been fixed. The rest of the small NSC occurring in the ventral median part, as well as in the other locations named above, do not possess so many PF^+

granules at any given time. The distinctive pair resemble Scharrer's (1955b) 'A' type cells. The large NSC may correspond to her 'B' type. All three size classes of NSC show definite cyclical activity. Minute intracellular vacuoles may be observed sometimes in all of the antero-median, antero-lateral and ventro-median NSC but these vacuoles are not as large as those of the larger ventro-lateral and larger ventro-median NSC. The large ventro-lateral cell lying close to the labial nerve possesses medium-sized vacuoles. Its cytoplasm stains grey or pink and may or may not contain a few PF^+ granules at a given stage in development, e.g. just before or just after the moult. Sometimes this cell contains an appreciable amount of PF^+ granules and at such times vacuoles are small and few or absent. Similar NSC were observed in the latero-lateral part of the ganglion.

The antero-median and antero-lateral groups are entirely composed of small NSC.

At certain times (see below) two ventro-median NSC, in nymphs and adults, afe filled with PF^+ granules and resemble some of the 3b and 3c NSC of locus 3. A general statement on the cycle of activity of any group or class of NSC can only be made if changes in these

cells are observed at short intervals of time over several instars. For example, in the case of NSC of the SOG Füller (1960) certainly missed the stage at which some of the cells, especially the small ventro-median pair, are full of purple granules, probably because he used mostly newly moulted nymphs and adults. According to the author's observations there is a marked depletion of PF^{+} purple granules in these two small ventro-median NSC in all freshly moulted nymphs and adults of both sexes. At this stage the cytoplasm of these cells stains light purple in colour. The amount of neurosecre-:tory material in these cells increases remarkably some time after the moult, the granules then staining a deep purple colour. For example, in last instar nymphs of both sexes, killed 1-9 hours after the moult (Fig. 107) and in adult males and females, sacrificed 1-12 hours after the moult (Fig. 108), these NSC have a light purple cytoplasm and contain only a few granules of the same colour. There is a marked increase in the number of these granules in last instar nymphs killed 89 and 90 days after the moult and in adult females killed 18 hours after the last moult (Fig. 109). In adult females killed 1 hour, 4 hours, 9 hours, 12 hours, 18 hours, 24 hours and 48 hours after the moult, the secretory cycle observed in this pair of small

Figure 107. Photomicrograph showing a small ventromedian neurosecretory cell (VMNSC) in the suboesophageal ganglion of a last instar ♀ nymph of P. americana killed just after the moult. NpSOG, neuropile of the suboesophageal ganglion. The cell has sparse PF^+ granules.

Figure 108. Photomicrograph showing small ventromedian neurosecretory cell (VMNSC) in the suboesophageal ganglion of an adult ♀ P. americana killed 1 hour after moulting. The cell has again sparse PF^+ granules.

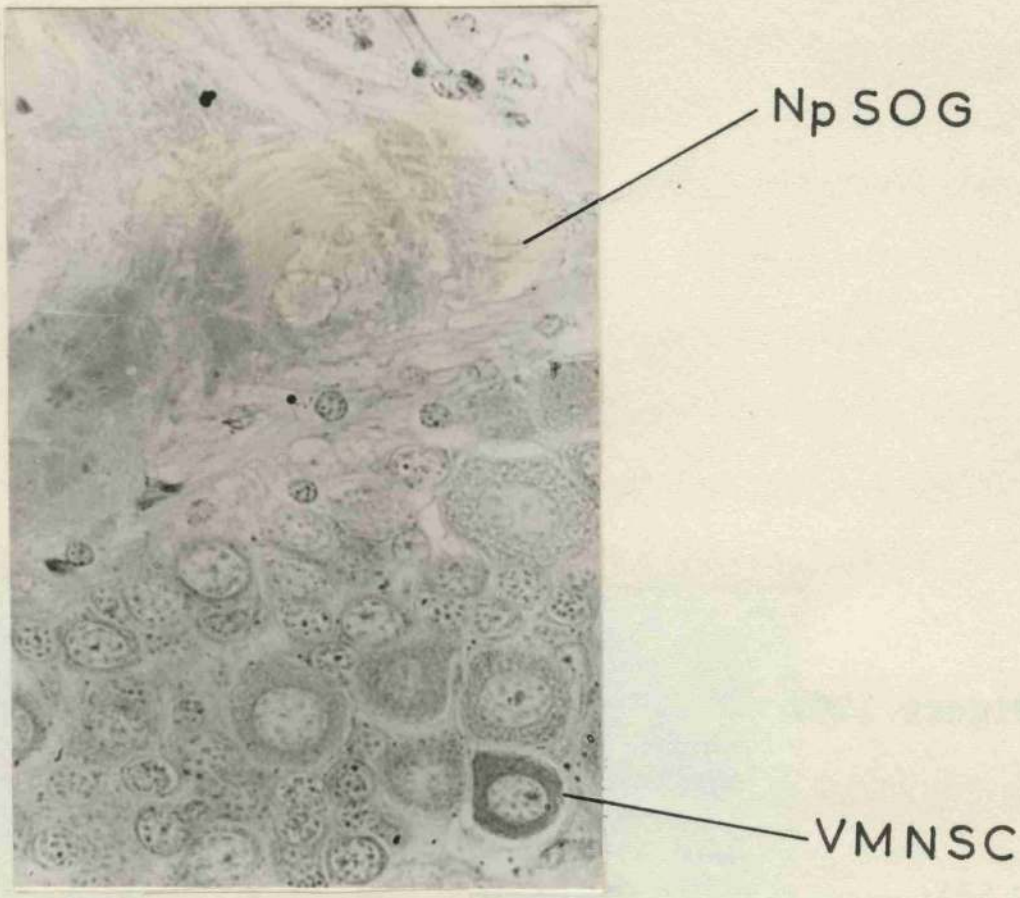


Figure 107.

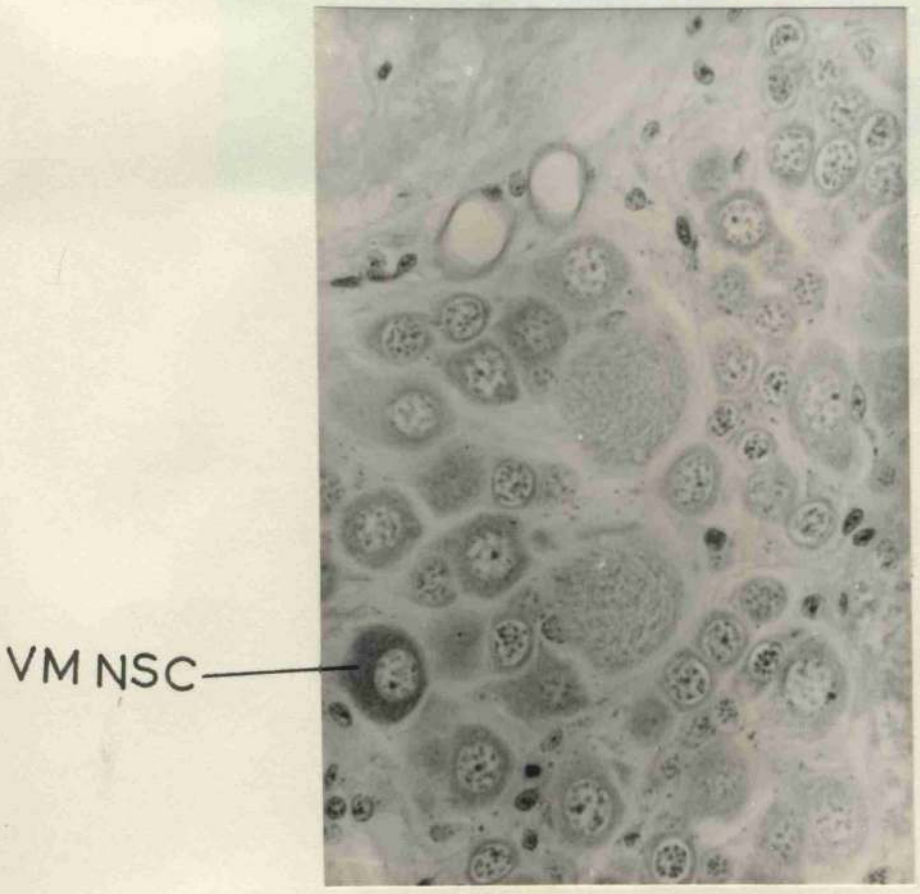


Figure 108.

Figure 109. Photomicrograph showing two small ventromedian neurosecretory cells (VMNSC) in the suboesophageal ganglion of an adult ♀ *P. americana* killed 18 hours after the moult. The cells have abundant PF^+ granules.

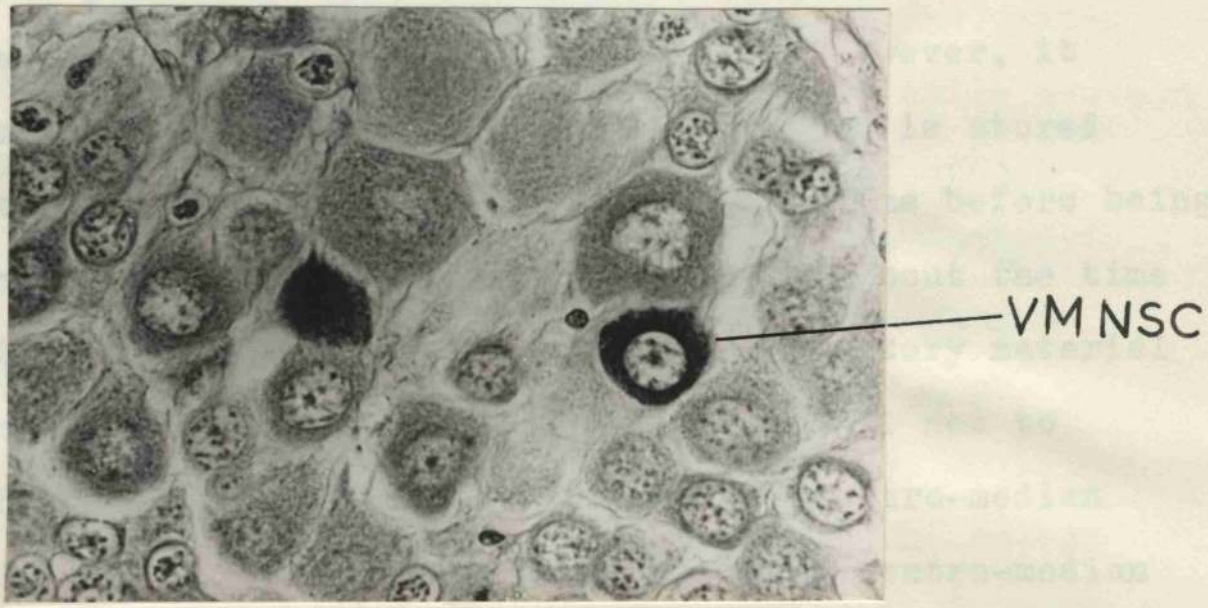


Figure 109.

ventro-median NSC is as follows: in the newly moulted specimens there is a notable lack of PF^+ material in these cells (Fig. 108). The amount of this material increases slightly at 4 hours, again at 9 hours and still more at 12 hours. This increase continues through the eighteenth and twenty-fourth hours, and by the forty-eighth hour these cells are packed with purple granules. Unfortunately, it could not be determined when discharge took place. However, it seems certain that neurosecretory material is stored in the perikaryon of these NSC for some time before being discharged. In nymphs depletion occurs about the time of the moult. Accumulation of neurosecretory material also occurs in the other NSC of the SOG but not to such a marked extent as in these small ventro-median cells. When full of PF^+ granules these ventro-median NSC are larger than the 3b NSC of locus 3 in the pars intercerebralis at the same stage in the secretory cycle. The fine PF^+ granules of the former do not form large aggregates whereas the granules of the 3b and 3c cells of locus 3 are 'coarser' and usually form distinct clumps as noted above.

The PF^+ granules in the large NSC of the SOG are not always scanty and peripheral as described by Scharrer

(1955b) in Leucophaea; sometimes many such granules can be seen throughout the cytoplasm. The relative amounts of neurosecretory products in the NSC of the SOG of late embryos and early nymphal instars is less than in the corresponding cells at similar stages of activity in last instar nymphs and adults. This is in line with Scharrer's (1955b) finding in Leucophaea.

According to Scharrer (1955b), in gonadectomized adult females of Leucophaea, the large 'B' cells contain abundant marginal green granules instead of scanty PF^{+} granules. She called these cells 'castration cells'. She also found some green bodies lying at random in and near the other neurons of the SOG. Without casting any doubt on her 'castration cells' and on her gonadectomy experiments, which the author has not performed, it is perhaps significant to note here that in slightly over counterstained (with indigo carmine) preparations the cytoplasm of the large NSC stains green and may contain either scanty or abundant green granules which may or may not be accompanied by vacuoles. The author could not find green bodies lying outside the cells as reported by Scharrer although in a few cases intercellular purple granules (whose significance is unknown) were observed.

Table 3

Age of *P. americana*	Total no. of NSC in the suboesophageal ganglion
42-day embryo	12
54-day embryo	4
First instar nymph killed ½ hr after hatching	2
First instar nymph killed 4 hr-12½ days after hatching	2
Third instar nymph ready to moult within a few days	30
Last instar ♂ nymph killed 89 days after moulting	10
Last instar ♀ nymph killed 90 days after moulting	12
Last instar ♀ nymph killed 1 hr after moulting	6
Last instar ♂ nymph killed 1 hr after moulting	8
Last instar ♀ nymph killed 9 hr after moulting	2
Freshly moulted adult females	8 - 12
Adult ♀ killed 9 hr after moulting	6
Adult ♀ killed 12 hr after moulting	14
Adult ♂ killed 12 hr after moulting	14
Adult ♀ killed 18 hr after moulting	8
Adult ♀ killed 24 hr after moulting	14
Adult ♀ killed 48 hr after moulting	21

Table 4. Summary of the observations on NSC in the central nervous system of cockroaches.

	Activity of NSC within the locus	Activity of NSC in various loci in relation to each other	Observations of previous workers on NSC of cockroaches				
			Scharrer, 1941, 1944, 1955b	Drescher, 1960	Miller, 1960	Willey, 1960	Pipa, 1961b
NSC in the protocerebrum							
paraconnectivus or ventral NSC — Locus 1 a	S	S	-	-	-	+ group 1	-
Locus 1 b	S						
Locus 1 c	S						
median NSC — Locus 2	S				-	+ group 2	-
Locus 3 a	S				-	-	-
Locus 3 b	A-s	S		-	+ A-β type, A-s	+ group 3	+ A-type, A-s
Locus 3 c	A-s				-		-
Locus 4 a	S	S	large and medium-sized NSC in the pars-intercerebralis	+ large NSC	-	+ group 4	-
Locus 4 b	S						
Locus 4 c	S			-			
Locus 4 d	S						
lateral NSC — Locus 5 a	S		-	-	-	+ group 5	-
Locus 5 b	S						
Locus 5 c	S						
NSC in the optic ganglion — Locus 6 a	S		-	-	-	-	-
Locus 6 b	S						
Locus 6 c	S						
Locus 6 d	S						
NSC in the deutocerebrum	A-s		-	-	-	-	-
NSC in the tritocerebrum	÷		-	-	-	-	-
NSC in the circum-oesophageal commissure							
a	÷		-	-	-	-	-
b	÷						
NSC in the frontal ganglion	A-s		-	-	-	-	-
NSC in the suboesophageal ganglion							
1. Antero-median group	S		ventro-lateral (A-type), ventro-median + (B-type) and latero-lateral (A-type)	only one large + ventro-lateral NSC	lateral, ventro-medial, and ventro-lateral. All A-β type (synchronous)	only one pair + of ventro-median NSC	-
2. Antero-lateral group	S						
3. Latero-lateral group	S						
4. Ventro-lateral group	S						
5. Ventro-median group	A-s						

S = synchronous + = recorded
A-s = asynchronous - = not recorded
+ = not worked out by the author as yet

Finally, according to Füller (1960) there are only eight NSC in the SOG of last instar nymphs and adults of P. americana but according to the author's observations the number of NSC in this ganglion, which can be seen at various times, varies but may be far in excess of a total of eight (see Table 3).

DISCUSSION

Neurosecretory material could not be demonstrated in the axons of locus 1 NSC either by PF or CHP staining procedure. The neurosecretory material may either be dispersed and passed along the axon in an unstained form or discharged peripherally as suggested by Matsumoto (1958). Intercellular PF^+ granules were found to be in close proximity to these cells. These granules may either be neurosecretory material discharged peripherally from the NSC, or may be gliosomes of the neighbouring glia cells as first described by Scharrer (1939). The former explanation is perhaps the more probable since these intercellular PF^+ granules occur very close to the periphery of the NSC and do not resemble the coarse gliosomes seen elsewhere in the brain. If the second explanation holds the destination of these PF^+ granules is not

known. Füller (1960) also describes and gives a photomicrograph of intercellular PF^+ secretory materail in the thoracic ganglion of P. americana. It is interesting to note here that 1b and 1c NSC of locus 1 resemble very closely B type NSC of the thoracic ganglion of B. orientalis described and photomicrographed by Füller (1960), but he did not find this cell type in the brain of either P. americana or B. orientalis. Pipa (1961b) also does not mention the para-connectivus NSC in P. americana. Willey (1961), however, in P. americana and Blaberus craniifer, describes these NSC as belonging to his group 1. These para-connectivus or ventral NSC of locus 1 were indeed observed by the author in P. americana and B. orientalis before Willey's published work was seen. However, these NSC, previously undescribed in the protocerebrum of the latter species, are recorded here for the first time.

The 3b NSC of locus 3 are undoubtedly the asynchronous A-α cells of Füller (1960) and the asynchronous A type NSC of Pipa (1961). Füller and Pipa did not describe the 3a and 3c type of NSC in the pars intercerebralis of P. americana. However, Füller's synchronous β- cells of A type which he described as occurring in the SOG, thoracic and first three abdominal ganglia and shown

in his figure 12, appear to be similar to the author's 3a synchronous NSC of locus 3.

Füller (1960) has described small C type cells occurring in the ventral nerve cord ganglia though he is not sure whether they are neurosecretory cells. These C cells as figured by Füller look similar to the author's 3c NSC of locus 3, though they differ in that the numerous PF^+ granules are in the form of clumps in the 3c NSC whereas in Füller's cells they are not. The neurosecretory granules of the author's 3c NSC stain both with PF and CH staining procedures while Füller reports that the neurosecretory granules of his C cells are only PF^+. However, Willey (1961) mentions the occurrence of small NSC (15μ) with PF^+ granules in his group 3 in the median pars intercerebralis. Thus the author and Willey are in agreement about the occurrence of small NSC in locus 3.

According to Willey (1961) the giant cells of his group 4 possess PF^+ granules in their axon-hillock, but he no longer regards these cells as neurosecretory. However, the author, in agreement with Drescher (1960) and Blest (1961), does regard these cells 4a as neurosecretory. The author, who studied 4a NSC of

locus 4 and other NSC of this locus from embryo to adult, finds that starting from the 42-day embryo, PF^+ or CH^+ granules could be seen in these NSC and these cells show signs of synchronous cyclic secretory activity. The author and Willey (1961) are in agreement about the presence of medium-sized (20-25μ) 4d NSC in locus 4, whose presence has not been recorded by Drescher (1960). The small cells (10-15μ) lying ventral to locus 4 NSC were not seen to possess either PF^+ or CH^+ granules as noted above, and are not regarded here as secretory. Drescher (1960) has stated that these small cells as well as the larger cells in this locus, corresponding to the author's 4a NSC, produce thoracotrophic hormone. After extirpating both large NSC (4a) and the more ventral smaller cells Drescher found that some nymphs still moulted, though this was delayed. Histological examination revealed that some of the small cells were left in place and he concluded that the smaller cells, as well as the giant cells, produce the thoracotrophic hormone which activates the prothoracic glands. Drescher mentions neither the time of extirpation, i.e. how long after the preceding moult the cells were extirpated, nor does he take into consideration the fact that the abrupt removal of NSC need not necessarily stop the

moulting of an insect in which the storage of neurosecre-:tory material in the corpora cardiaca plays an important role. In fact, Johansson (1957, 1958) in Oncopeltus has shown that the fifth stage nymphs whose NSC were completely extirpated "as early as four hours after the preceding moult were capable of moulting".

It must be stated here that the author is unable to agree with the rigid classification of neurosecretory cells into A and B types (see Introduction), for the following reasons.

(a) It was not found possible to selectively stain cells into A and B types with paraldehyde fuchsin.

(b) The neurosecretory granules in one cell may well stain pink, light purple, reddish, or dark purple at different stages of the secretory cycle.

(c) Phloxinophilic granules could not be demonstrated with CHP staining.

(d) The observations on the asynchronous activity of the 3b and 3c NSC of

> locus 3 indicate that little significance can be assigned to the size of the cell and the amount of neurosecretory material present at any given time. If this is overlooked there is a danger that cells of the same type at different stages of activity may be thought to be of different types. The author's observa-:tions on NSC in the central nervous system of cockroaches and a comparison of these with observations of other workers are summarised in Table 4.

If all the cells described above are neurosecretory one is faced with the conclusion that a considerable proportion of the nerve cells in the brain of cockroaches are secretory. Other investigators have encountered the same problem. In polychaetes, Scharrer (1937) found that nearly half the nerve cells in the supra-oesophageal ganglion of Aphrodite are neurosecretory, while Clark (1956) has stated that neurosecretory cells occupy nearly three-quarters of the supraoesophageal ganglion. of Nephtys. Similarly in a bug, Oncopeltus, Johansson (1958) has found that many neurons in the central nervous system show secretory activity. All

neurons have the ability to secrete but, as Johansson implies, the more specialized neurons either show an increase in secretory activity (e.g. neurons in the pars intercerebralis), or have lost all signs of neurosecretion (e.g. globuli cells and motor cells).

CONCLUSIONS

Despite the fact that cockroaches are ubiquitous insects and familiar and useful animals in teaching and research laboratories, there are a number of anatomical details which have not been described by the previous investigators. For example, the tritocerebral lobes are united extracerebrally, not only by the short thick post-oesophageal commissure but also by a second large slender anterior post-oesophageal commissure. Also it is commonly known that the protocerebral lobes are connected to the corpora cardiaca (CC) by the nervi corporis cardiaci interni and externi (NCCI and NCCII) but, apart from the author, only two contemporary workers (Drescher, 1960; Willey, 1961) have noted the presence of a pair of NCCIII linking the tritocerebral lobes to the CC. These corpora are themselves united by a dorsal commissure. Anterior to this structure lie the intra-aortal regions and posterior to it the extra-aortal regions of the CC. The two corpora allata (CA) are also inter-:connected by an allatal commissure and, in addition to the NCAI which contains axons which originate in the protocerebrum and are incorporated in the NCC as far as the CC, the CA also receive fibres from the suboesophageal ganglion in the NCAII.

The suboesophageal ganglion is also in nervous connec-:tion with the prothoracic glands, while a side branch of each NCAII also innervates these glands.

It has long been known that the brain, stomatogastric nervous system and retrocerebral endocrine organs are connected by nerves but the degree to which these elements are interconnected has not until recently been appreciated. The descriptions of these nerves given in this work are believed to be the most detailed which have yet been pro-:duced and, apart from the author, only one contemporary worker (Willey, 1961) has attempted such a description.

The embryonic development of the brain of the American cockroach has been studied in great detail and only a few of the more significant observations can be dealt with here. For example, the optic ganglion of the cockroach, like the rest of the brain, is formed <u>in embryo</u> by neuroblasts which divide unequally to give rise to large cells (neuroblasts) and small cells (ganglion mother cells). The large cells continue to divide unequally while each ganglion mother cell certainly divides (equally) to produce two daughter cells which become ganglion cells. Sometimes dividing daughter cells were also observed. Thus some of the daughter cells may also divide before they are transformed into the ganglion cells.

The 'intraganglionic thickening' is the formation centre of the optic ganglion. The other meristematic cells which take part in the formation of the optic ganglion lie in the region of the lamina ganglionaris.

The perineurium and glia cells have a common origin. Both are derived from cells of mesodermal origin, princi-:pally those of the cephalic coelomic rudiments, which actually invade the developing brain.

After the forty-second day of embryonic development the structure of the brain is morphologically similar to that of the nymph and it subsequently remains essentially the same throughout post-embryonic life. The only changes which occur during post-embryonic development are a steady increase in the number of neurons (globuli cells of the corpora pedunculata, cells of the optic ganglion, cells in other parts of the brain) and an increase in the cytoplasmic volume of individual neurons. There is also a gradual increase in the size of the association centres and the neuropile and an increase in the number of perineurium and glia cells.

Since, at times, neurosecretory cells show obvious signs of senescence, yet the number of such cells is maintained, there must be some centre of recruitment and

this is thought to lie in the internal calyx of the corpus pedunculatum since globuli cells originating in this location, which have been displaced outwards by their successors, appear to acquire more cytoplasm than the neighbouring globuli cells and to become neurosecretory cells.

There is no reason to suppose that neurosecretion starts in cockroach embryos as early as Jones (1956) suggests it does in locusts. The first sign of neurosecretion in the brain and suboesophageal ganglion, detectable by the ordinary light microscope in appropriately stained sections, occurs in the 42-day embryo. Only one phase of neurosecretion occurs during embryogenesis of P. americana, in late embryos when the brain is fully formed and the neurons are well defined and resemble those of first instar nymphs. Median, lateral and ventral neurosecretory cells in the protocerebrum, first observable in the 42-day embryo, occur throughout nymphal development and in the adult, and show cyclical activity. Neurosecretory cells also occur in the optic ganglion, the deutocerebrum, probably in the tritocerebrum and circum-oesophageal connectives, frontal ganglion and the suboesophageal ganglion. In fact, a large proportion of the neurons in the central nervous system (excluding the thoracic and abdominal ganglia which

the author has not studied as yet) show signs of secretory activity. The activity of some types of cells is asynchronous while that of the others is synchronous.

The neurosecretory cells in the suboesophageal ganglion first seen in the 42-day embryo, occur also in nymphs and adults, and show cyclical secretory activity. There are five groups of neurosecretory cells in this ganglion, namely:

1. Antero-median;
2. ventro-median;
3. antero-lateral;
4. latero-lateral;
5. ventro-lateral

Neurosecretory material is present in the NCCI and NCCII and is transmitted thereby to the CC and the CA. Neurosecretory material occurs for the first time in the CC and CA of the 42-day embryo. These glands undergo cyclical changes during late embryonic and post-embryonic development. Cyclical changes in the neuroendocrine system are related in the embryo to eclosion and in nymphs to moulting and related processes. That such a cycle occurs first in the embryo prior to eclosion is an indication that there is a physiological similarity between the processes of eclosion and moulting.

The histological evidence indicates that the activity of the CA is restrained to some degree by the neurosecretory cells of the protocerebrum at every moult. There is also histological evidence of such a restraint being applied in the late embryo before eclosion. The rate of change of the CA is probably most rapid and certainly most noticeable at the penultimate and final moults. The CA of last instar nymphs and adults, killed immediately after ecdysis, possess abundant intercellular neurosecretory granules and are thought to be inactive since they are small, their cytoplasmic volume is low and hence the small nuclei appear to be concentrated or crowded together. However, after no more than one hour most of the neurosecretory material disappears from the CA and the glands now increase in size. The volume of cytoplasm increases and hence the nuclei, which are now larger, appear to be dispersed or less concen-:trated. These changes are generally regarded as indicative of activity in the CA. The relationship between the neuroendocrine elements and the CA can only be shown by careful timing, i.e. by killing a large number of insects at short intervals of time before and after ecdysis. It must therefore, finally, be emphasized that neurosecretory activity and neurosecretory cycles must not be sought, or observed, at intervals of several days or even of one day - as is common practice - but at the minimum time intervals which are practical.

REFERENCES

ARVY, L. BOUNHIOL, J.J. & GABE, M. (1953) Déroulment de la neuro-sécrétion protocerebrale chez Bombyx mori L., cours du developpement post-embryonnaire. C.R. Acad. Sci., Paris, 236, 627-629.

ARVY, L. & GABE, M. (1952a) Données histophysiologiques sur la neuro-sécrétion chez quelques Ephemeropteres. La Cellule, 55, 203-222.

ARVY, L. & GABE, M. (1952b) Données histophysiologiques sur les formations endocrines retrocérébrales de quelques Odonates. Ann. Sci. nat. (Zool.), 14, 345-372.

ARVY, L. & GABE, M. (1953a) Données histophysiologiques sur la neuro-sécrétion chez les Insectes Paléoptéres. Z. Zellforsch., 38, 591-610.

ARVY, L. & GABE, M. (1953b) Données histophysiologiques sur les glandes endocrines cephaliques de Brachyptera risi Morton (Plécoptère). Arch. Zool. exp. gén., 90, 105-112.

ARVY, L. & GABE, M. (1953c) Particularités histologiques des glandes endocrines rétrocérébrales chez Tenebrio molitar L. C.R. Acad. Sci., Paris, 237, 844-846.

ARVY, L. & GABE, M. (1954a) The intercerebralis-cardiacum-allatum system of some Plecoptera. Biol. Bull. Woods Hole, 106, 1-14.

ARVY, L. & GABE, M. (1954b) Données histophysiologiques sur la neuro-sécrétion chez les insectes Paléoptéres. Pubbl. Staz. Zool. Napoli, 24, Suppl., 54-55.

BACCETTI, B. (1955) Ricerche sulla fine struttura del perilemma nel sistema nervosa degli Insetti. Redia, 40, 197-212.

BACCETTI, B. (1956) Ricerche preliminari sui connettivi e sulle membrane basali degli Insetti. Ibid, 41, 75-104.

BADEN, V. (1937) Enbryology of the nervous system in the grasshopper Melanoplus differentialis (Acrididae, Orthoptera). J. Morph., 60, 159-190.

BALDUS, K. (1924) Untersuchungen über die Bau und Funktionen des Gehirns der Larvae, und Imago von Libellen (Odonata). Z. wiss. Zool. Leipzig, 121, 557-620.

BARGMANN, W. (1949) Über die neurosekretorische Verknupfung von Hypothalamus und Neurohypophyse. Z. Zellforsch., 34, 610-634.

BARTH, R.H. (1961) Hormonal control of sex attractant production in the Cuban cockroach. Science, 133, 1598-1599.

BAUER, V. (1903) Zur innern Metamorphose des Centralnervensystems der Insekten (Vorläufige Mitteilung). Zool. Anzeiger, 25, 655-656.

BAUER, V. (1904) Zur innern Metamorphose des Centralnervensystems der Insekten. Zool. Jb. (Anat.), 20, 123-152.

BLEST, A.D. (1961) Some modifications of Holmes's silver method for insect central nervous system. Quart. J. micr. Sci., 102, 413-417.

BODENSTEIN, D. (1951) Endocrine control of metamorphosis with special reference to Holometabola. Trans. 9th int. Congr. Ent., 58-62.

BODENSTEIN, D. (1953a) Studies on the humoral mechanisms in growth and metamorphosis of the cockroach, Periplaneta americana L. I. Transplantations of integumental structures and experimental parabiosis. J. exp. Zool., 123, 189-232.

BODENSTEIN, D. (1953b) Studies on the humoral mechanisms in growth and metamorphosis of the cockroach, Periplaneta americana L. II. The function of the prothoracic gland and the corpus cardiacum. J. exp. Zool., 123, 413-433.

BODENSTEIN, D. (1954) Endocrine mechanisms in the life of insects. Recent Progress in Hormone Research, 10, 157-178.

BOUNHIOL, J.J., GABE, M. & ARVY, L. (1953) Données histophysiologiques sur la neuro-sécrétion chez Bombyx mori L., et sur ses rapports avec les glandes endocrines. Bull. biol., 87, 323-333.

BOUNHIOL, J.J., GABE, M. & ARVY, L. (1954) Données histophysiologiques sur la neuro-sécrétion chez Bombyx mori L., et sur ses rapports avec les glandes endocrines. Pubbl. Staz. Zool. Napoli, 24, Suppl., 52-53.

BRETSCHNEIDER, F. (1914) Über die Gehirne der Küchenschabe und des Mehlkäfers. Jen. Zeit. Naturwiss., 52, 269-362.

BURTT, E.T. (1938) On the corpora allata of dipterous Insects. Proc. Roy. Soc., B, 126, 210-223.

CAMERON, M. (1953) Secretion of an orthodiphenol in the corpus cardiacum of the Insect. Nature, Lond., 172, 349-350.

CARLISLE, D.B. & KNOWLES, F.G.W. (1953) Neurohaemal organs in Crustaceans. Nature, Lond., 172, 404-405.

CASPARI, E. & PLAGGE, E. (1935) Versuche zur Physiologie der Verpuppung von Schmetterlingsraupen. Naturwissenschaften, 23, 751-752.

CAZAL, P. (1948) Les glandes endocrines rétrocérébrales des insectes (étude morphologique). Bull. biol., Suppl., 32, 1-227.

CAZAL, P. & GUERRIER, Y. (1946) Reserches sur les glandes endocrines rétrocérébriodiennes des Insectes. I. Étude morphologique chez les Orthoptères. Arch. Zool. exp. gén., 84, 303-334.

CHADWICK, L.E. (1955) Moulting of roaches without prothoracic glands. Science, 121, 435.

CHADWICK, L.E. (1956) Removal of prothoracic glands from the nymphal cockroach Periplaneta americana L. J. exp. Zool., 131, 291-306.

CLARK, R.B. (1959) The neurosecretory system of the supraoesophageal ganglion of Nephtys (Annelida, Polychaeta).
Zool. Jb. (Anat.), 68, 395-424.

DAY, M.F. (1940) Neurosecretory cells in the ganglia of Lepidoptera.
Nature, Lond., 145, 264.

DEROUX-STRALLA, D. (1948) Recherches expérimentales sur le rôle des 'glandes ventrales' dans la mue et la métamorphose, chez Aeshna cyanea Müll. (Odonata).
C.R. Acad. Sci., Paris, 227, 1277-1278.

DRESCHER, W. (1960) Regenerationversuche am Gehirn von Periplaneta americana L., unter Berücksichtigung von Verhaltensänderung und Neurosekretion.
Z. Morph. Ökol. Tiere, 48, 576-649.

DUPONT-RAABE, M. (1956) Mise en évidence, chez les phasmides d'une troisième paire de Nervi corporis cardiaci, voie possible de cheminement de la substance chromactive tritocérébrale vers les corpora cardiaca.
C.R. Acad. Sci., Paris, 243, 1240-1243.

DUPONT-RAABE, M. (1957) Les mécanismes de l'adaptation chromatique chez les insectes.
Arch. Zool. exp. gén., 94, 61-294.

DUPONT-RAABE, M. (1958) Quelques aspects des phénomènes de neuro-sécrétion chez les phasmides.
In II int. Symp. Neurosekretion. Ed. W. Bargmann, B. Hanström, B. & E. Scharrer, pp. 85-90. Berlin: Springer.

DU PORTE, E.M. (1959) "Manual of Insect Morphology." New York: Reinhold. 224 pp.

EASTHAM, L.E.S. (1930) The embryology of Pieris rapae. Organogeny. Phil. Trans., B, 219, 1-50.

EHNBOM, K. (1948) Studies on the central and sympathetic nervous system and some sense organs in the head of neuropteroid insects. Opusc. Ent., Suppl. 8, 1-162.

ENGELMANN, F. (1957) Die Steuerung der Ovarfunktion bei der ovoviviparen Schabe Leucophaea maderae (Fabr.). J. ins. Physiol., 1, 257-278.

ENGELMANN, F. (1959) The control of reproduction in Diploptera punctata (Blattaria). Biol. Bull. Woods Hole, 116, 406-419.

ENGELMANN, F. (1960) Mechanisms controlling reproduction in two viviparous cockroaches (Blattaria). Ann. N.Y. Acad. Sci., 89, 516-536.

EPHRUSSI, B. & BEADLE, G.W. (1936) A technique of transplantation for Drosophila. Amer. Nat., 70, 218-225.

FEYER, (1912) Die postembryonale Entwicklung des Zentralen Nervensystems von Agelastica alni. Dissertation, Berlin. (Quoted from B. Scharrer, 1939.)

FRAENKEL, G. (1935) A hormone causing pupation in the blowfly Calliphora erythrocephala. Proc. Roy. Soc., B, 118, 1-12.

FLÖGEL, J. (1878) Über den einheitlichen Bau des Gehirns in den verschiedenen Insektenordnungen. Z. wiss. Zool., Suppl. 30, 556-592.

FRASER, A. (1957) An investigation of the phenomenon of diapause in the larva of Lucilia caesar L. (Diptera: Tachinidae). Ph.D. Thesis, University of Glasgow, 175 pp.

FUKUDA, S. (1940a) Induction of pupation in the silkworm by transplanting the prothoracic gland. Proc. imp. Acad. Japan, 16, 414-416.

FUKUDA, S. (1940b) Hormonal control of moulting and pupation in the silkworm. Proc. imp. Acad. Japan, 16, 417-420.

FUKUDA, S. (1944) The hormonal mechanism of larval moulting and metamorphosis in the silkworm. J. Fac. Sci. Tokyo Univ., Sec. IV, 6, 477-532.

FUKUDA, S. (1951a) Factors determining the production of non-diapause eggs in the silkworm. Proc. imp. Acad. Japan, 27, 582-586.

FUKUDA, S. (1951b) The production of the diapause eggs by transplanting the suboesophageal ganglion in the silkworm. Proc. imp. Acad. Japan, 27, 672-677.

FUKUDA, S. (1952) Function of the pupal brain and suboesophageal ganglion in the production of non-diapause and diapause eggs in the silkworm. Ann. Zool. Japan, 25, 149-155.

FUKUDA, S. (1953a) Determination of voltinism in the uni-voltine silkworm. Proc. imp. Acad. Japan, 29, 381-384.

FUKUDA, S. (1953b) Determination of voltinism in the multi-voltine silkworm. Proc. imp. Acad. Japan, 29, 385-388.

FUKUDA, S. (1953c) Alteration of voltinism in the silkworm following transection of pupal oesophageal connectives. Proc. imp. Acad. Japan, 29, 389-391.

FÜLLER, H.B. (1960) Morphologische und experimentelle Untersuchungen über die neurosekretorischen Verhältnisse im Zentralnervensystem von Blattiden und Culiciden. Zool. Jb. (Anat.), 69, 223-250.

GABE, M. (1953a) Sur quelques applications de la coloration par la fuchsin-paraldehyde. Bull. Micr. appliquée, 3, 153-162.

GABE, M. (1953b) Données histologiques sur les glandes endocrines céphaliques de quelques Thysanoures. Bull. Soc. zool. France, 78, 177.

GABE, M. (1954) La neurosécrétion chez les invertebres. Ann. Biol., 30, 5-62.

GELDIAY, S. (1959) Neurosecretory cells in ganglia of the roach Blaberus craniifer. Biol. Bull. Woods Hole, 117, 264-274.

GOMORI, G. (1941) Observations with differential stains on human islets of Langerhans. Amer. J. Path., 17, 395-406.

GOMORI, G. (1950) Aldehyde fuchsin; a new stain for elastic tissue. Am. J. Clin. Path., 20, 665-666.

GRANDORI, L. (1955) Anello di Weismann, metamorfosi e neurosecrezioni in Calliphora erythrocephala Meig., e Musca domestica L. Bollettino del Laboratorio di Zoologica Generale e Agraria "Filippa Silvestri", Portici, 33, 198-237.

GRANDORI, R. & GRANDORI, L. (1954) Sistema capillare nel complesso neuroendocrino di Musca domestica L., e Calliphora erythrocephala Meig. Rivista di Parassitologia, 15, 437-448.

HALBWACHS, M.C., JOLY, L. & JOLY, P. (1957) Resultats d'implantations de "glandes ventrales" a Locusta migratoria L. J. ins. Physiol., 1, 143-149.

HALLE, B. (1905) Über den allgemein Bauplan des Tracheansyncerebrums. Arch. mikr. Anat., 65, 181-279.

HANDORN, E. (1937) An accelerating effect of normal "ring glands" on puparium formation in lethal larvae of Drosophila melanogaster Proc. Nat. Acad. Sci. Wash., 23, 478-484.

HANDORN, E. & NEEL, J. (1938) Der hormonale Einfluss der Ringdrüsse (corpus allatum) auf die Puparіumbildung bei Fliegen. Arch. Entw. Mech. Org., 138, 281-304.

HANSTRÖM, B. (1928) "Vergleichende Anatomie des Nervensystems der wirbellosen Tiere." Berlin: Springer. 628 pp.

HANSTRØM, B. (1938) Zwei Probleme betreffs der hormonalen Lokalisation im Insektenkopf. Acta Univ. Lund, N.F., Avd. 39, 1-17.

HANSTRØM, B. (1940) Inkretorische Organe, Sinnesorgane und Nervensystem des Kopfes einiger niederer Insektenordnungen. K. svenska Vetensk Akad. Handl. 18, 3-265.

HANSTRØM, B. (1953) Neurosecretory pathways in the head of crustaceans, insects and vertebrates. Nature, Lond., 171, 72-73.

HARKER, J.E. (1960a) The effect of perturbations in the environmental cycle on the diurnal rhythm of activity of Periplaneta americana L. J. exp. Biol., 37, 154-163.

HARKER, J.E. (1960b) Internal factors controlling the suboesophageal ganglion neurosecretory cycle in Periplaneta americana L. J. exp. Biol., 37, 164-170.

HARKER, J.E. (1960c) Endocrine factors in insect circadian rhythms. Symposium on Quantitative Biology, 25, 279-287.

HARNACK, M. von & SCHARRER, B. (1956) A study of the corpora allata of gonadectomized Leucophaea maderae (Blattaria). Anat. Rec., 125, 558.

HASEGAWA, K. (1951) Studies on voltinism in the silkworm, Bombyx mori L., with special reference to the organs concerning determination of voltinism. Proc. imp. Acad. Japan, 27, 667-677.

HASEWAGA, K. (1957) The diapause hormone of the silkworm, Bombyx mori. Nature, Lond., 179, 1300-1301.

HERLANT-MEEWIS, H. & PAQUET, L. (1956) Neurosécrétion et mue chez Carausius morosus Brdt. Ann. Sci. nat. (Zool.), 18, 163-168.

HESS, A. (1958) The fine structure of nerve cells and fibres, neuroglia, and sheaths of the ganglion chain in the cockroach (Periplaneta americana L.). J. Biophys. Biochem. Cytol., 4, 731-742.

HEYMONS, R. (1895) "Die Embryonalentwicklung von Dermapteren und Orthopteren." Jena: Gustav Fischer.

HEYMONS, R. (1899) Beiträge zur Morphologie und Entwicklungsgeschichte der Rhynchoten. Nova Acta Leop.-carol, 74, 352-455.

HIGHNAM, K.C. (1958) Activity of the brain corpora cardiaca system during pupal diapause 'break' in Mimas tibiae (Lepidoptera). Quart. J. micr. Sci., 99, 73-88.

HIGHNAM, K.C. (1961) The histology of the neurosecretory system of the adult female Desert Locust, Schistocerca gregaria. Quart. J. micr. Sci., 102, 27-38.

HIS, W. (1889) Die Neuroblasten und deren Enstehung im embryonalen Mark. Abhandl. d.k. sächs. Gesell. 15 (quoted from Wheeler, 1893).

HODGSON, E.S. & GELDIAY, S. (1959) Experimentally induced release of neurosecretory materials from roach corpora cardiaca. Biol. Bull. Woods Hole, 117, 275-283.

HOFER, B. (1887) Untersuchungen über den Bau der Speicheldrüsen und des dazu gehörenden Nervenapparats von Blatta.
Nova Acta Leop.-carol, 51, No. 6, 349-395.

HOLMGREN, E. (1960) Weitere Mitteilung über die 'Saftkanälchen' der Nervenzellen.
Anat. Anz., 18, 149-153.

HOYLE, G. (1952) High blood potassium in insects in relation to nerve conduction.
Nature, Lond., 169, 281.

IMMS, A.D. (1951) "A general textbook of entomology". 8th edition.
London: Methuen.

JAWLOWSKI, H. (1948) Studies on the insect brain.
Ann. Univ. Marie Curie-Sklodowska Lublin Polina, 3, 1-30.

JOHANNSON, S.A. (1957) Neurosecretion in the Milkweed Bug, Oncopeltus fasciatus (Dallas) (Heteroptera: Lygaeidae).
In II int. Symp. Neurosekretion. Ed. W. Bargmann, B. Hanström, B. & E. Scharrer, pp. 98-101. Berlin: Springer.

JOHANNSON, S.A. (1958) Relation of nutrition to endocrine-reproductive functions in the milkweed bug, Oncopeltus fasciatus (Dallas) (Heteroptera: Lygaeidae).
Nytt Magasin for Zoologi, 7, 1-132.

JOHANSSEN, O.A. & BUTT, F.H. (1941) "Embryology of insects and myriapods."
New York: McGraw-Hill. 453 pp.

JONES, B.M. (1956) Endocrine activity during insect embryogenesis. Function of the head glands in locust embryos (Locustana pardalina, and Locusta migratoria, Orthoptera). J. exp. Biol., 33, 174-185.

KLOOT, W.G. van der (1960) Neurosecretion in insects. Ann. Rev. Ent., 5, 35-52.

KOBAYASHI, M. (1957) Quoted from K.C. Highnam, 1961. Bull. Sericul. exp. Stat. Tokyo, 15, 181 (in Japanese with English summary).

KOBAYASHI, M. & BURDETTE, J.W. (1961) Effect of brain hormones from Bombyx mori on metamorphosis of Calliphora erythrocephala. Proc. Soc. exp. Biol. Med., 107, 240-242.

KOPEC, S. (1917) Experiments on metamorphosis in insects. Bull. int. Acad. Cracovie (B), 57-60.

KOPEC, S. (1922) Studies on the necessity of the brain for the inception of insect metamorphosis. Biol. Bull. Woods Hole, 42, 322-342.

KÖPF, H. (1957) Über Neurosekretion bei Drosophila. I. Zur Topographie und Morphologie neurosekretorische Zentren bei der Imago von Drosophila melanogaster. Biol. Zbl., 76, 28-42.

KOROTNEFF, A. (1885) Die Embryologie der Gryllotalpa. Z. wiss. Zool., 41, 570-604.

KUHN, A. & PIEPHO, H. (1936) Über hormonale Wirkung bei der Verpuppung der Schmetterlinge. Nachricht Ges. Wiss. Göttingen, Mathphys. Klasse, N.F. Fachgr. VI, Nachr. a.d. Biol., 2, 141-154.

LERMA, B. de (1933) Corpori frangei degli ortotteri. Prova sicura della eistenza di glandole endocrine negli artropodi. Atti Acad. Lincei Ren. Classi Sci. fis. mat. nat., 17, 1105-1108.

LERMA, B. de (1937) Observazioni sul sistema endocrino degli insetti (corpora allata e corpi faringei). Archiv. Zool. Ital., 24, 339-368.

LERMA, B. de (1956) Corpora cardiaca et neurosécrétion protocérébrale chez le Coleoptere Hydrous piceus L. Ann. Sci. nat. (Zool.), 18, 235-249.

LUSCHER, M. & ENGELMANN, F. (1960) Histologische und experimentelle Untersuchungen über die Auslösung der Metamorphose bei Leucophaea maderae. J. ins. Physiol., 5, 240-258.

LUSCHER, M. & SPRINGHETTI, A. (1960) Untersuchungen über die Bedeutung der corpora allata für die Differenzierung der Kasten bei der Termite Kalotermes flavicollis F. J. ins. Physiol., 5, 190-212.

MALLIS, A. (1954) "Handbook of Pest Control." 2nd edition. New York: MacNair-Dorland.

MATSUMOTO, K. (1954) Neurosecretion in the thoracic ganglion of Eriocheir japonicus. Biol. Bull. Woods Hole, 106, 60-68.

MATSUMOTO, K. (1956) Migration of the neurosecretory products in the thoracic ganglion of the crab, Chionoectes opilio. Biol. J. Okayama Univ., 2, 137-146.

MATSUMOTO, K. (1958) Morphological studies on neurosecretion in crabs. Biol. J. Okayama Univ., 4, 103-176.

NABERT, A. (1913) Die Corpora allata der Insekten. Z. wiss. Zool., 104, 181-358.

NAYAR, K.K. (1954) The structure of the corpus cardiacum of Locusta migratoria. Quart. J. micr. Sci., 95, 245-250.

NAYAR, K.K. (1955a) Studies on the neurosecretory system of Iphita limbata Stal. I. Distribution and structure of the neurosecretory cells of the nerve ring. Biol. Bull. Woods Hole, 108, 296-307.

NAYAR, K.K. (1955b) Neurosecretory cells in insects. Agra Univ. J. Res., 4, 419-422.

NAYAR, K.K. (1955c) Neurosecretory cells in the larvae of the gall midge. Current Science, 24, 90-91.

NAYAR, K.K. (1956) Studies on the neurosecretory system of Iphita limbata Stal. III. The endocrine glands and the neurosecretory pathways in the adult. Z. Zellforsch., 44, 697-705.

NAYAR, K.K. (1960) Studies on the neurosecretory system of Iphita limbata Stal. VI. Structural changes in the neurosecretory cells induced by changes in water content. Z. Zellforsch., 51, 320-324.

NEDER, R. (1959) Allometrische Wachstum von Hirnteilen bei drei verschieden grossen Schaben. Zool. Jb. (Anat.), 77, 411-464.

NELSON, J.A. (1915) "The embryology of the Honey Bee." Princeton University Press. pp. 1-282.

NELSON, J.A. (1924) Morphology of the Honey Bee larva. J. Agric. Res., 28, 1167-1213.

NESBITT, H.H.J. (1941) A comparative morphological study of the nervous system of the Orthoptera and related orders. Ann. Ent. Soc. Amer., 34, 51-81.

NEWTON, E.T. (1879) On the brain of Blatta orientalis. Quart. J. micr. Sci., 19, 340-356.

NOIROT, C. (1957) Neurosécrétion et sexualité chez la Termite a cou jaune (Calotermes flavicollis F.). C.R. Acad. Sci. Paris, 245, 743-745.

NUSBAUM, J. (1883) Vorläufige Mitteilung über die Chorda der Arthropoden. Z. Anz., 6, 291-295.

NUSBAUM, J. (1886) The embryonic development of the cockroach. In Miall and Denny 'The Structure and Life-history of the Cockroach', 181-204.

NUSBAUM, J. & FULINSKI, B. (1906) Über die Bildung der Mitteldarmanlage bei Phyllodromia (Blatta). Z. Anz., 30, 362-381.

NUSBAUM, J. & FULINSKI, B. (1909) Zur Entwicklungsgeschichte des Darmdrüsenblattes bei Gryllotalpa vulgaris. Z. wiss. Zool., 93, 308-348.

NYST, R.H. (1942) Structure et rapports du système nerveux, du vaisseau dorsal et des annexes cardiaques chez Dixippus morosus Br. Ann. Soc. Roy. Zool. Belg., 73, 150-164.

OZBAS, S. (1957) Morphological and histological studies on the corpora allata and corpora cardiaca in Orthoptera. Commun. Fac. Sci. Ankara, Serie C, Sci. nat., 8, 19-44.

OZBAS, S. & HODGSON, E.S. (1958) Action of insect neurosecretion upon the central nervous system in vitro and upon behaviour. Proc. Nat. Acad. Sci., 44, 825-830.

OZEKI, K. (1958a) Effect of corpus allatum on development of male genital organs of the Earwig, Anisolabis maritima. Sci. Pap. Coll. Gen. Educ. Univ. Tokyo, 8, 69-75.

OZEKI, K. (1958b) Neurosecretion of the Earwig, Anisolabis maritima. Sci. Pap. Coll. Gen. Educ. Univ. Tokyo, 8, 85-92.

PANOV, A.A. (1957) Bau des insekten Gehirns während der postembryonalen Entwicklung. I. Mushroom bodies. Ent. rev. Acad. Sci. U.S.S.R., 36, 269-284. (In Russian with German summary.)

PFEIFFER, I.W. (1936) The effects of removal of the corpora allata on egg production in the grasshopper Melanoplus differentialis. Proc. Soc. exp. Biol. Med., 34, 385. (Quoted from A. Fraser, 1957.)

PFEIFFER, I.W. (1939) Experimental study of the function of the corpora allata in the grasshopper, Melanoplus differentialis. J. exp. Zool., 82, 439-461.

PFEIFFER, I.W. (1945) The influence of the corpora allata over the development of nymphal characters in the grasshopper Melanoplus differentialis. Trans. Conn. Acad. Arts Sci., 36, 489-515.

PFLUGFELDER, O. (1936-37) Vergleichendanatomische, experimentalle und embryologische Untersuchungen über das Nervensystem und die Sinnesorgane der Rhynchoten. Zoologica Stuttgart, 34, 1-102.

PFLUGFELDER, O. (1937) Bau, Entwicklung, und Funktion der Corpora allata und cardiaca von Dixippus morosus Br. Z. wiss. Zool., 149, 477-512.

PFLUGFELDER, O. (1941) Tatsachen und Probleme der Hormonforschung bei Insekten. Arch. für die allgemeine Fragen der Lebensforschung, 15, 197-235.

PFLUGFELDER, O. (1947) Die Ventraldrüssen und andere inkretorische Organe des Insektenkopfes. Biol. Zbl., 66, 211-235.

PFLUGFELDER, O. (1948) Volumetrische Untersuchungen an den Corpora allata der Honigbiene. Biol. Zbl., 67, 223-241.

PFLUGFELDER, O. (1952) "Entwicklungsphysiologie der Insekten." Leipzig. 328 pp.

PIPA, R.L. (1961a) Studies on the hexapod nervous system. III. Histology and histochemistry of cockroach neuroglia. J. comp. Neurol., 116, 15-22.

PIPA, R.L. (1961b) Studies on the hexapod nervous system. IV. A cytological and cytochemical study of neurons and their inclusion in the brain of a cockroach, Periplaneta americana L. Biol. Bull. Woods Hole, 121, 521-534.

PLAGGE, E. (1938) Weitere Untersuchungen über das Verpuppungshormon bei Schmetterlingen. Biol. Zbl., 58, 1-12.

POLICE, G. (1910) Sulla discussa natura di alcune parti del sistema nervoso viscerale degli Insetti. Arch. zool. Ital., 4, 287-314.

POSSOMPÈS, B. (1950) Rôle du cerveau au cours de la métamorphose de Calliphora erythrocephala Meig. C.R. Acad. Sci. Paris, 231, 594-596.

POSSOMPÈS, B. (1953) Recherche expérimentale sur le determinisme de la métamorphose de Calliphora erythrocephala Meig. Arch. Zool. exp. gén., 89, 203-364.

POULSON, D.F. (1950) Development of the principal ectodermal organs. The nervous system. In 'The Biology of Drosophila', edited by M. Demerac. New York. pp. 209-224.

POWER, M.E. (1943) The brain of Drosophila melanogaster. J. Morph., 72, 517-559.

RICHARDS, O.W. & DAVIES, R.G. (1957) "A general textbook of entomology." 9th edition. London: Methuen. 831 pp.

ROMANES, G.J. (1950) The staining of nerve fibres in paraffin sections with silver. J. Anat., 84, 104-115.

ROONWAL, M.L. (1936) Studies on the embryology of the African migratory locust, Locusta migratoria migratorioides. I. The early development, with a new theory of multiphased gastrulation among insects. Phil. Trans., B, 226, 391-421.

ROONWAL, M.L. (1937) Studies on the embryology of the African migratory locust, Locusta migratoria migratorioides. II. Organogeny. Phil. Trans., B, 227, 175-244.

ROTH, L.M. & STAY, B. (1961) Oocyte development in Diploptera punctata (Eschscholtz) (Blattaria). J. ins. Physiol., 7, 186-202.

SANCHEZ, D. (1933) Contribution à la connaissance de la structure des corps fungiformes (calices) et de leurs pédicules chez la blatte commune (Blatta orientalis). Trav. Lab. Rech. Biol. Univ. Madrid, 28, 149-185.

SCHARRER, B. (1937) Über sekretorisch tätige Nervenzellen bei wirbellosen Tieren. Naturwissenschaften, 25, 131-138.

SCHARRER, B. (1939) The differentiation between neuroglia and connective tissue sheath in the cockroach Periplaneta americana L. J. comp. Neurol., 70, 77-87.

SCHARRER, B. (1941) Neurosecretory cells in the central nervous system of cockroaches. J. comp. Neurol., 74, 93-108.

SCHARRER, B. (1946a) The role of the corpora allata in the development of Leucophaea maderae (Orthoptera: Blattaria). Endocrinology, 38, 35-45.

SCHARRER, B. (1946b) The relationship between the corpora allata and reproductive organs in adult Leucophaea maderae. Endocrinology, 38, 46-55.

SCHARRER, B. (1946c) Section of nervi corporis cardiaci in Leucophaea maderae.
Endocrinology, 96, 577.

SCHARRER, B. (1948a) The prothoracic glands of Leucophaea maderae (Orthoptera).
Biol. Bull. Woods Hole, 95, 186-198.

SCHARRER, B. (1948b) Hormones in insects.
In 'The Hormone', Ed. G. Pincus & K.V. Thimann, 1, 121-158. New York: Academic Press.

SCHARRER, B. (1951) The storage of neurosecretory material in the corpus cardiacum.
Anat. Rec., III, 554-555.

SCHARRER, B. (1952a) Über neuroendocrine Vorgänge bei Insekten.
Pflügers Archiv, 255, 154-163.

SCHARRER, B. (1952b) Neurosecretion. XI. The effects of nerve section on the intercerebralis-cardiacum-allatum system of the insect Leucophaea maderae.
Biol. Bull. Woods Hole, 102, 261-272.

SCHARRER, B. (1952c) The effect of the interruption of neurosecrotory pathway in the insect Leucophaea maderae.
Biol. Bull. Woods Hole, 112, 386-387.

SCHARRER, B. (1953) Comparative physiology of invertebrate endocrines.
Ann. Rev. Physiol., 15, 457-472.

SCHARRER, B. (1955a) Hormones in invertebrates.
In 'The Hormone', Ed. G. Pincus & K.V. Thimann, 3, Ch. 2, 57-95. New York: Academic Press.

SCHARRER, B. (1955b) Castration cells in the central nervous system of Leucophaea maderae (Blattaria). Trans. N.Y. Acad. Sci. [2], 17, 520-525.

SCHARRER, B. (1958) Neuroendocrine mechanisms in insects. In II int. Symp. Neurosekretion, Ed. W. Bargmann, B. Hanström, B. & E. Scharrer, 79-84. Berlin: Springer.

SCHARRER, B. (1961) Functional analysis of the corpus allatum of the insect Leucophaea maderae with the electron microscope. Biol. Bull. Woods Hole, 121, 370.

SCHARRER, B. & HARNACK, M. von (1958) Histophysiological studies on the corpus allatum of Leucophaea maderae. I. Normal life cycle in male and female adults. Biol. Bull. Woods Hole, 115, 508-520.

SCHARRER, B. & HARNACK, M. von (1961) Histophysiological studies on the corpus allatum of Leucophaea maderae. III. The effect of castration. Biol. Bull. Woods Hole, 121, 193-208.

SCHARRER, B. & SCHARRER, E. (1944) Neurosecretion. VI. A comparison between the intercerebralis-cardiacum-allatum system of insects and the hypothalamo-hypophyseal system of vertebrates. Biol. Bull. Woods Hole, 87, 242-251.

SCHARRER, E. & BROWN, S. (1961) Neurosecretion. XII. The formation of neurosecretory granules in the earthworm, Lubricus terrestris L. Z. Zellforsch., 54, 530-540.

SCHARRER, E. & SCHARRER, B. (1945) Neurosecretion. Physiol. Rev., 25, 171-181.

SCHARRER, E. & SCHARRER, B. (1954a) Neurosekretion. Handb. mikr. Anat. Menschen. Ed. W. v. Möllendorff and W. Bargmann, VI, 5, 953-1066.

SCHARRER, E. & SCHARRER, B. (1954b) Hormones produced by neurosecretory cells. Recent Progress in Hormone Research, 10, 183-226.

SCHRADER, K. (1938) Untersuchungen über die Normalentwicklung des Gehirns und Gehirntransplantationen bei der Mehlmatte Ephestia kühniella. Zeller nebst einigen Bemerkungen über das corpus allatum. Biol. Zbl., 58, 52-90. (Quoted from B. Scharrer, 1939).

SHARAN, R.K. & SAHANI, S.L. (1960) Provisional embryonic cuticles of Dysdercus cingulatus (Hemiptera: Pyrrhocoridae). Ann. ent. Soc. Amer., 53, 538-541.

SMITH, D.S. & WIGGLESWORTH, V.B. (1958) Collagen in the perilemma of insect nerve. Nature, Lond., 183, 127-128.

SNODGRASS, R.E. (1935) "Principles of insect morphology." New York: McGraw-Hill Book Co. Inc. 667 pp.

SNODGRASS, R.E. (1943) The feeding apparatus of biting and disease carrying flies: A wartime contribution to medical entomology. Smithsonian misc. Collec., 104, 1-51.

SPATZ, H. (1953) Das Hypophysen-Hypothalamus-System in seiner Bedeutung für die Fortpflanzung. Anat. Anz., (Suppl.) 100, 48-86.

STEEDMAN, H.F. (1947) Ester wax, a new embedding medium. Quart. J. micr. Sci., 88, 123-133.

STRINDBERG, H. (1913) Embryologische Studien an Insekten. Z. wiss. Zool., 106, 1-227.

STUM-ZOLLINGER, E. (1957) Histological study of regenerative processes after transection of the nervi corporis cardiaci in transplanted brains of the cecropia silkworm (Platysamia cecropia L.). J. exp. Zool., 134, 315-325.

STUTINSKY, F. (1952) Étude du complexe rétro-cérébral de quelques insectes avec l'hématoxyline chromique. Bull. Soc. zool. France, 77, 61-67.

THOMSEN, E. (1940) Relation between corpus allatum and ovaries in adult flies (Muscidae). Nature, Lond., 145, 28.

THOMSEN, E. (1952) Functional significance of the neurosecretory brain cells and the corpus cardiacum in the blowfly Calliphora erythrocephala Mg. J. exp. Biol., 29, 137-172.

THOMSEN, E. (1954) Studies on the transport of neurosecretory material in Calliphora erythrocephala Mg. by means of ligaturing experiments. J. exp. Biol., 31, 322-330.

THOMSEN, E. & MØLLER, I.B. (1959) Neurosecretion and intestinal proteinase activity in an insect, Calliphora erythrocephala Mg. Nature, Lond., 183, 1401-1402.

THOMSEN, M. (1954a) Observations on the cytology of neurosecretion in various insects (Diptera and Hymenoptera). Pubbl. Staz. Zool. Napoli, 24 (Suppl.), 46-47.

THOMSEN, M. (1954b) Neurosecretion in some Hymenoptera. Det Konglige Danske Videnskabernes Selskab Biol. Skrifter, 7, 1-24.

TIEGS, O.W. & MURRAY, F.V. (1938) The embryonic development of Calandra oryzae. Quart. J. micr. Sci., 80, 159-284.

TOMAYA, K. (1902) Contributions to the study of silkworms. 1. On the embryology of the silkworm. Bull. Coll. Agric. Japan, 5, 73-118.

VANNUCCI, M. (1953) The function of the corpora cardiaca of a grasshopper, Preliminary note. Dusenia, 4, 439-442.

VIALLANES, H. (1890) Sur quelques Points de l'Histoire du Developpement embryonnaire de la Mante religieuse. Rev. Biol. du Nord de la France, 2, No. 12. (Quoted from Wheeler, 1893.)

VIALLANES, H. (1891) Developpement de la Mante religieuse. Ann. Sci. Nat. Zool., 11, 283-328. (Quoted from Baden, 1937.)

WEBER, H. (1952) Morphologie, Histologie und Entwicklungsgeschichte der Articulaten. Fortschr. Zool. (N.F.), 9, 18-231.

WEYER, F. (1935) Über drüsenartige Nervenzellen im Gehirn der Honigbiene, Apis mellifica L. Zool. Anz., 112, 137-141.

WHEELER, W.M. (1889) The embryology of Blatta germanica and Doryphora decemlineata. J. Morph., 3, 291-386.

WHEELER, W.M. (1891) Neuroblasts in the arthropod embryo. J. Morph., 4, 337-344.

WHEELER, W.M. (1893) A contribution to insect embryology. J. Morph., 8, 1-142.

WHITMAN, C.O. (1878) The embryology of Clepsine. Quart. J. micr. Sci., 18. (Quoted from W.M. Wheeler, 1893.)

WHITMAN, C.O. (1887) A contribution to the history of germ layers in Clepsine. J. Morph., 1. (Quoted from W.M. Wheeler, 1893.)

WIESMANN, R. (1926) "Zur Kenntnis der Anatomie und Entwicklungsgeschichte von Carausius morosus." Jena. (Quoted from O. Pflugfelder, 1952, in 'Entwicklungsphysiologie der Insekten, 5, 182. Leipzig.)

WIGGLESWORTH, V.B. (1933) The physiology of the cuticle and of ecdysis in Rhodnius prolixus Stal (Hemiptera); with special reference to the function of the oenocytes and of the dermal glands. Quart. J. micr. Sci., 76, 269-318.

WIGGLESWORTH, V.B. (1934) The physiology of ecdysis in Rhodnius prolixus Stal (Hemiptera). II. Factors controlling moulting and 'metamorphosis'. Quart. J. micr. Sci., 77, 191-222.

WIGGLESWORTH, V.B. (1936) The function of the corpus allatum in the growth and reproduction of Rhodnius prolixus Stal (Hemiptera). Quart. J. micr. Sci., 79, 91-121.

WIGGLESWORTH, V.B. (1939) Source of moulting hormone in Rhodnius. Nature, Lond., 144, 753.

WIGGLESWORTH, V.B. (1940) The determination of characters at metamorphosis in Rhodnius prolixus (Hemiptera). J. exp. Biol., 17, 201-222.

WIGGLESWORTH, V.B. (1948) The functions of the corpus allatum in Rhodnius prolixus Stal (Hemiptera). J. exp. Biol., 25, 1-14.

WIGGLESWORTH, V.B. (1951) Hormones and metamorphosis, with special reference to hemimetabolic insects. Trans. 9th int. Congr. Ent., 2, 51-57.

WIGGLESWORTH, V.B. (1952a) The thoracic gland in Rhodnius prolixus Stal. (Hemiptera) and its role in moulting. J. exp. Biol., 29, 561-570.

WIGGLESWORTH, V.B. (1952b) Hormone balance and control of metamorphosis in Rhodnius prolixus Stal (Hemiptera). J. exp. Biol., 29, 620-631.

WIGGLESWORTH, V.B. (1953) The origin of sensory neurons in an insect, Rhodnius prolixus Stal (Hemiptera). Quart. J. micr. Sci., 94, 93-112.

WIGGLESWORTH, V.B. (1954a) "The physiology of insect metamorphosis." Cambridge: University Press. 152 pp.

WIGGLESWORTH, V.B. (1954b) Neurosecretion and the corpus cardiacum of insects. Pubbl. Staz. Zool. Napoli, 24 (Suppl.), 41-45.

WIGGLESWORTH, V.B. (1956) The haemocytes and connective tissue formation in an insect, Rhodnius prolixus Stal (Hemiptera). Quart. J. micr. Sci., 97, 89-98.

WIGGLESWORTH, V.B. (1957a) The action of growth hormones in insects. Symp. Soc. exp. Biol., 11, 204-227.

WIGGLESWORTH, V.B. (1957b) The use of osmium in fixation and staining of tissues. Proc. Roy. Soc., B, 147, 185-199.

WIGGLESWORTH, V.B. (1959a) The histology of the nervous system of an insect, Rhodnius prolixus Stal (Hemiptera). II. The central ganglia. Quart. J. micr. Sci., 100, 299-313.

WIGGLESWORTH, V.B. (1959b) A simple method for cutting sections in the 0.5 to 1μ range, and for sections of chitin. Quart. J. micr. Sci., 100, 315-320.

WIGGLESWORTH, V.B. (1960) The nutrition of the central nervous system in the cockroach, Periplaneta americana L. The role of perineurium and glia cells in the mobilization of reserves.
J. exp. Biol., 37, 500-512.

WIGGLESWORTH, V.B. (1961) Some observations on the juvenile hormone effect of farnesol in Rhodnius prolixus Stal (Hemiptera).
J. ins. Physiol., 7, 73-78.

WILLEY, R.B. (1961) The morphology of the stomodaeal nervous system in Periplaneta americana L., and other Blattaria.
J. Morph., 108, 219-262.

WILLEY, R.B. & CHAPMAN, G.B. (1960) The ultrastructure of certain components of the corpora cardiaca in orthopteroid insects.
J. Ultrastructure Res., 4, 1-14.

WILLIAMS, C.M. (1946) Insect diapause: the role of the brain in the production and termination of pupal dormancy in the giant silkworm, Platysamia cecropia.
Biol. Bull. Woods Hole, 90, 234-243.

WILLIAMS, C.M. (1947) Physiology of insect diapause. II. Interaction between the pupal brain and prothoracic glands in the metamorphosis of the giant silkworm, Platysamia cecropia.
Biol. Bull. Woods Hole, 93, 89-98.

WILLIAMS, C.M. (1948a) Physiology of insect diapause. III. The prothoracic glands in the cecropia silkworm, with special reference to their significance in embryonic and post-embryonic development. Biol. Bull. Woods Hole, 94, 60-65.

WILLIAMS, C.M. (1948b) Extrinsic control of morphogenesis as illustrated in metamorphosis of insects. Growth Symp., 12, 61-74.

WILLIAMS, C.M. (1952) Physiology of insect diapause. IV. The brain and prothoracic glands as an endocrine system in the cecropia silkworm. Biol. Bull. Woods Hole, 103, 120-138.

WILLIAMS, C.M. (1959) The juvenile hormone. I. Endocrine activity of the corpora allata of the adult cecropia silkworm. Biol. Bull., 116, 323-338.