STUDIES CONCERNING NEUROSECRETION IN THE KHAPRA BEETLE; TROGODERMA GRANARIUM EVERTS.

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November, 1964.

A B S T R A C T

The Khapra beetle, Trogoderma granarium. Everts is a pest of stored products. Larvae of late instars enter dormancy under unfavourable conditions.

The anatomy, histology and growth of the central nervous system are studied. Growth of the ganglia is attributed to both increase in size and number of the cells.

The neuro-endocrine system in the six instars of non-dormant larvae, dormant larvae, pupa and the adult is described. Three types of neurosecretory cells are found in the brain and the suboesophageal ganglion. These cells show cytological changes throughout the insect life.

The effect of the external environment on the activity of the neurosecretory system in the larva is studied. Food and temperature influence the synthesis and release of neurosecretory material. The phases of dormancy and metamorphosis are controlled by different levels of hormonal activity.

Autoradiographic investigations were carried out. They give results which agree with the histological inferences.

The anatomy, histology and growth of the reproductive system in unmated and mated males and females are described. The details of the formation of resorption bodies in the ovarioles of non-ovipositing females and the formation of the "corpora lutea" are described. The activity of the neurosecretory system is correlated with maturation, copulation, oviposition and spermatogenesis.

To elucidate the rôle of the components of the neuro-endocrine system, cauterisation, implantation and ligature experiments and farnesol treatments were carried out. Experiments revealed that the phase of dormancy, metamorphosis, egg and testecular development, oviposition are controlled by different levels of hormonal activity. Experiments also indicate a close inter-relationship between the different components of the neuro-endocrine system.

Intermediate forms and abnormalities are caused by upsets in the hormone balance.

The role of the male in inducing maturation and oviposition in the female is studied. Maturation in either the male or the female is

affected by the presence of the opposite sex.

The effects of sublethal doses of pyrethrum and DDT on the neuro-endocrine system and the physiological consequences are described.

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I. INTRODUCTION

The field of neurosecretion has developed so rapidly in the past 15 years that it has now become interrelated with a host of other disciplines from neuroanatomy to developmental physiology, from metabolism and biochemistry to behaviour and ecology.

Various reviews of the literature on neurosecretion have been given by Scharrer and Scharrer (1954), Bodenstein (1954) Wigglesworth (1954, 1959, 1964), Welsh (1959), Van der Kloot (1960, 1962), in the Proceedings of Three International Symposia on Neurosecretion (1953, 1957, 1961) and Tahewaki (1962). Considered together, these papers cover the whole field of neurosecretion.

Evidence for the presence of hormones of neuro-endocrine origin in insects was first obtained by Kopeč in 1922. He removed the brain of a freshly moulted last instar larva of the moth Lymantria dispar and found that the larva failed to pupate; but it was not until 1935 that Weyer, working on the brain of the honey bee, Apis mellifera showed that the neurosecretory cells of the pars intercerebralis might be the source of the hormone. Since Weyer's time, with the development of new and better differential stains, more and more cells and cell types have been described in other families and orders of insects. It now appears likely that their occurrence is universal in the Insecta if not in all higher Metazoa.

The criterion for distinguishing neurosecretory cells from other neurones has been generally adopted from Scharrer's (1954) definition, namely that 'Neurosecretory cells are nerve cells which show cytological evidence of secretion'. Johansson (1958) and Ladduwahetty (1962) have found secretory inclusions in the cell which are probably motor neurones. To avoid confusion, Van der Kloot (1960) suggested that the term 'neuro-endocrine' cells should be used to designate the neurosecretory cells which elaborate and release hormones. Berns (1962) pointed out that a variety of inclusions present in the neurones may mimic neurosecretory material, such as neuromelanin, stored metabolites and pigmented materials of unknown

significance. He defined a neurosecretory cell as one in which '...the signs of secretory activity can be related to the production of chemical agents with measurable physiologic effects, agents definable as hormones'.

Two main types of neurosecretory cells were first designated as A and B - cells according to their staining reactions by Scharrer (1954) These cells that stain purple with Paraldehyde Fuchsin (PF) and blue-black with Chrome-haematoxylin/Phloxine (CHP) are designated as A cells. The B cells are phloxinophil and stain red with CHP and red or bluish green with PF. This classification has since been accepted and followed by many workers; e.g. Thomsen (1954) on several species of Hymenoptera, Nayar (1955) on Iphita limbata, Fraser (1959) on Lucilia caesar, and Highnam (1961) on Schistocerca gregaria. There have also been other reports of other types of neurosecretory cells e.g. C and D, or III and IV. In these cells, however, there is no uniformity in classification. For example, the C cells present in Dermestes described by Ladduwahetti (1962) may not necessarily be analogous to the C cells previously described in Oncopeltus by Johansson (1959). It seems that standardisation of staining techniques is much to be desired in comparative histological investigation of this nature.

A conspicuous character of the neurosecretory cells is their axons, along which the neurosecretory material is transported, commonly to the organs of storage and release. It has now been established that in all the insects studied the neurosecretory products from the A cells of the brain are conducted along axonal routes which leave the brain by the nervi corporis cardiaca and enter the corpora cardiaca. The corpus cardiacum is found to be the organ of storage of secretion from the brain in many insects, viz., Leucophaea maderae (Scharrer 1951, 1952), Iphita limbata (Nayar, 1956), Carabus nemoralis (Klug 1958, 1959), Blaberus craniifer (Hodgson and Geldiay 1959) and Schistocerca gregaria (Highnam, 1961). In some insects, however, the material does not appear to be stored in the corpora cardiaca, but passes along the corporis allati to the corpora allata for storage and release: e.g., Dermestes maculatus (Ladduwahetty 1962). A few other workers have also reported the presence of brain secretory material

in the corpora allata, among them Khan (1962), who has shown that careful timing is necessary to demonstrate the presence of material in these organs in embryonic stages of Periplaneta americana. The corpora cardiaca and allata have also been shown to produce their own hormones e.g., Wigglesworth (1934 - 1954), Thomsen (1952), Bodenstein (1954) and Highnam (1962).

Most reports on the occurrence and characteristic of neurosecretory cells have come from workers who confined themselves to a study of only one growth stage of an insect, e.g., larva, pupa or adult and devoted their attention to the brain, e.g., Wigglesworth (1940) Scharrer (1941, 1955, 1956), Thomsen (1952, 1954), Nayar (1953, 1955, 1956), Johansson (1957, 1958) and Highnam (1961, 1962) With respect to centres other than the brain, relatively little is known except for the suboesophageal ganglion which is often examined at the same time as the brain and is known to contain neurosecretory cells, e.g., Scharrer (1941), Rehm (1955), Nayar (1955), Brandenburg (1956), Harker (1955, 1960), Kopf (1957a,b), Johansson (1958), Fuller (1960), Kirchner (1960), Panov (1962) and Delphin (1963) Reports on the presence of neurosecretory cells in the ventral nerve cord have been made by Day (1940), Clements (1956), Kobayashi (1957), Johansson (1958), Geldioy (1959), Fraser (1959), Fuller (1960), Maddrell (1962) and Delphin (1963).

While some workers have concerned themselves only with histological descriptions of neurosecretory systems, others have tried to correlate the changes occurring in them with various physiological states of insects and/to confirm their conclusions experimentally The general conclusion reached is that growth, metamorphosis and reproduction are under the control of hormones produced by various components of the endocrine system However, there is a need for further comprehensive studies in this important field for there is a lack of information on certain aspects, e.g., the relationship between neurosecretion and reproduction in the male and on the metamorphosis of the neurosecretory system -- and there are some contradictory statements to resolve these will be discussed later

This work is believed to be the first attempt to study the neurosecretion in all ganglia of the central nervous system, from a histological and physiological standpoint in all the stages of an

insect, including nondormant larvae, dormant larvae, pupae and adults. The species involved is the 'Khapra beetle' Trogoderma granarium Everts.

Trogoderma granarium, an insect of economic importance, has been selected for this study because of the facultative diapause or quiescence of the larva and the possible neurosecretory basis of this phenomenon, its short life which enabled me to study the metamorphosis of the neurosecretory system and finally the ease of culture that ensured its availability in large numbers.

From an economic view point, Trogoderma granarium is one of the more important members of the species of the Dermestid beetles in the world which are regarded as pests. It is commonly found in grain stores and in malt, barley and dried milk. A survey in England showed that T. granarium here is confined to vegetable products and that it occurred on malt and to a lesser extent on wheat, barley, kibbled rice, rice, rice flour, peas, beans and ground nuts. In Germany, the species was recorded in malt-houses (Voelket 1914). The insect has also been studied in Japan (Nakayama, 1931) because of damage caused by the larvae feeding on stored rice.

The biology of T. granarium, including the effect of temperature, humidity and food on its life cycle, was studied by many writers of whom Hadaway (1956) and Burges (1956, 1957, 1959, 1960, 1962) are the most important. The excellent work of the latter consisted of detailed studies of the biology of this insect under favourable and unfavourable environmental conditions. In the favourable environments, the cycle from egg to adult requires only one month (Hadaway, 1956. Burges, 1957) whereas under unfavourable environmental conditions the insect may enter facultative diapause and remain in this state for more than four years (Burges, 1959).

The principal histological and experimental problems presented by T. granarium are the thick cuticle and the tufts of barbed hairs that cover the larval skin, which made fixation and embedding rather difficult. Also, the minuteness of the insect generally and the head in particular and the difficulty with which the latter can be dissected and operated upon. However, the application of new techniques and the use of recently developed entomological equipment have made study of even the smallest insects feasable.

This work has been divided into twelve main sections

1. Anatomy and histology of the neurosecretory system in the six instars of nondormant larvae.
2. Anatomy and histology of the neurosecretory system in dormant larvae.
3. Experimental investigations on neurosecretion and metamorphosis in the nondormant larva.
4. Experimental investigations on neurosecretion and dormancy in dormant larvae.
5. Anatomy and histology of neurosecretory system in the pupa.
6. Experimental investigations on neurosecretion and metamorphosis in the pupa.
7. Morphological and histological studies of the neurosecretory system in male and female adults.
8. The occurrence of cytological and morphometric changes in the neurosecretory system in different physiological phases of both male and female adults.
9. Morphology, histology and growth of the reproductive system throughout the male and female life history.
10. Histological and experimental studies on neurosecretion, in relation to reproduction.
11. Studies of the role of the male in inducing maturation of the female.
12. The effect of sub-lethal doses of pyrethrum and DDT on the neurosecretory system, on growth and reproduction.

II GENERAL METHODS AND TECHNIQUES

Because of the differences between the tuft-haired integument of the larvae, the thin delicate cuticle of the pupa and the hard thick cuticle of adults, various fixatives were used for different periods to achieve the best results.

a - Dissecting technique

Insects were kept in Pampels fluid (Imms, 1939) for at least 24 hours; and in practice it was found that it could be kept in this fluid for months with the tissues soft and in good condition for subsequent dissection. Material was then passed through 70%, 50% alcohol and water. Dissection was made under water using very fine pins, a jeweller's forceps and a high-powered dissecting microscope. The dissection was stained with Delafield's Haematoxylin containing a few drops of glacial acetic acid, thus avoiding over-staining. Different views of whole-mounted preparations were studied in Farrant's medium.

For vivisected material, dissection was carried out under Ringer's solution. Saline media for Periplaneta (Pantin, 1948) proved satisfactory for most purposes and the living tissues remained in a good condition for many hours.

b - Histological techniques

1. Neurosecretory system

Aqueous Bouin, Carnoy, Susa, Mukerji's fluid (Ranendra, 1953) and Alcoholic Bouin (Duboscq - Brasil) were used for fixing. Aqueous Bouin was found satisfactory in fixing first and second instar larvae and the pupae for 6 - 8 and 12 hours respectively. However, it did not give satisfactory results when used for fixing the other stages. Carnoy gave good results, but it caused excessive shrinkage,

particularly when used for fixing young instars and pupae. Mukerji's fluid was tried for fixing the adults only; it softened the cuticle satisfactorily, but it caused damage to internal systems. Susa was found unsatisfactory in fixing old instars and adults. The best results were achieved by using Alcoholic Bouin for all stages. Care was taken that the material was not over-fixed. Fixing periods ranged from 6 to 24 hours and fixative was always used warm (40°C.) Dehydration was accomplished by an alcohol and the best results were obtained when butyl alcohol was used.

Paraffin wax with melting points of 58°C and 62°C and also Ester wax were used for embedding. To achieve the best results, embedding was carried out under vacuum for late instars and adults. Sections were cut transversely and longitudinally at various thicknesses from 0.5 μ to 4 μ.

The stains employed were:

1. Gomori's (1950) Aldehyde-fuchsin (Paraldehyde Fuchsin) as modified by Halmi (1952) and Gabe (1953).
2. Gomori's (1941) Chrome-haematoxylin/Phloxine.
3. Heidenhain's Azan/Aniline blue (Pantin, 1948).
4. Alcian blue/Phloxine, after potassium permanganate oxidation (Delphin, 1963).

The first three stains gave successful results, but not the fourth. The best results were obtained by applying Paraldehyde fuchsin (PF). This was used in all the studies. Chrome-haematoxylin/Phloxine (CHP) and Azan were only employed when confirmation and comparison were required.

2. Reproductive system

Hot Duboscq-Brasil or Smith's Formol Bichromate were employed for fixing the adults. Both gave successful results, but in using the latter the abdomens were isolated just before fixing. Xylene was avoided as a clearing agent, and methyl benzoate was employed instead. Paraffin wax with a melting point of 58°C. was used, and sections were cut at 4 μ. The stain employed was Heidenhain's Iron Alum Haematoxylin, with Eosi as a counter-stain if required. For

staining vivisected material, e.g., spermatheca, vesiculae seminales, and testes, it was first squashed in a drop of Ringer's solution on a slide and then stained with Aceto-carmine.

c - Morphometric studies

1. Determination of cell number

The following general formula by Engelman (1957) was first used:

$$N = \frac{N' \times V}{A(T+2r)}$$

where N = total number of cells in each orga
N' = number of cells counted in A;
A = total area of sections selected for cellular counts;
V = volume of each organ;
T = thickness of section;
2r = average cellular diameter

However, as sections were cut at 2μ it was found that it would be more accurate if the cells were counted in each section. The mean cell diameter was calculated. The count was then corrected using a factor determined by section thickness (2μ) divided by mean cell diameter, thus correcting for count duplication of cells in serial sections.

2. Determination of volume

Outlines of successive sections of the organs were drawn by the aid of a camera lucida. The area of the outline drawing was measured with a planimeter graduated in centimetres. The area was then converted into μ^2 by a conversion graph. The volume of the section was calculated by multiplying the area by (T) thickness of the section. The total volume of the organ was the summation of the calculated volumes of the sections.

The nuclear volumes were calculated on the assumption that the nuclei were spheroids. The general formula for the volume was then:

$$V = \frac{4}{3} \pi \frac{a^2 b}{8}$$

where a and b were two diameters and (a) is the larger.

d - Cauterisation of neurosecretory cells in the brain

Mature non-dormant and dormant larvae and adults of both sexes were used.

Two methods were employed for cauterising the neurosecretory cells of the brain. The first was based on estimating the thickness of the cuticle and the site of the region in the brain which contains the neurosecretory cells. Thus cauterisation was made through the cuticle without opening the head. The second method was based on chilling the larva before the operation and cauterisation was made through an opening in the head capsule.

Although the second method is more accurate, it is interesting to know that both methods gave the same satisfactory results.

The first method

From dissected and sectioned specimens, the thickness of the cuticle and site of the region of the brain which contains the neurosecretory cells could be estimated.

The operating stage was of plasticine spread over a piece of glass. A thin pillow of plasticine was arranged upon the base. The insect was placed so that its head was on the pillow and was fastened to the operating stage by a thin pad of plasticine. The pressure of the latter induced the insect to protrude its head. However, as the insect tends to hide its head, a thin pin was inserted under the neck. Thus the head was firmly in position.

A battery operated micrautery built by Mr. J.W. Siddorn was used, and the operation was performed by the aid of a dissecting microscope with magnification 44 times. The fine platinum

wire was bent in such a way that the distance across its tip measured approximately 60 μ. The hot platinum wire was then introduced gently to cauterise the region of the brain containing the neurosecretory cells.

Practice attempts of cauterisation were made on insects and the time taken for each cautery was recorded. Insects which did not survive were fixed and sectioned to determine the cause of death, which was generally found to be due to damage to the aorta. Finally it was found that a temperature from 400° - 600° is highly satisfactory for cautery, and 2 - 2.5 seconds were enough to remove the region which contained the medial and lateral groups of neurosecretory cells.

The use of wax to seal the wound, as conducted by all the previous investigators, was found to be unsatisfactory. Even a minute drop of wax on the tip of a fine entomological pin, when applied, spreads over the whole head and concealed the mouthparts. Thus a new way of sealing the wound was devised. This was the application of the synthetic adhesive 'Durofix', which did not spread but accumulated and sealed the wound satisfactorily. However, before applying it, any extruded haemolymph was absorbed by a small piece of filter paper to ensure adhesion by the glue.

The second method

The same micrautery and operating platform were employed. However, the larva was chilled for 10 minutes and then placed with its head on the plasticine pillow, held in place with a plasticine pad. No pin was inserted under the head, in this operation, as the larva was motionless, and did not hide its head. A small window was cut in the posterio-dorsal region of the head by means of very fine pins, made specially for this purpose, and one pair of fine forceps. The brain was carefully exposed and great care was taken not to damage the aorta and the large lateral trunks. The hot cautery was then introduced very gently to destroy the neurosecretory cells. In this method, one second was found to be enough to remove all the cells. The window was then closed and the wound was sealed in the same way described above.

In operating on the adult, chilling was found unsatisfactory. Also, in several cases, the original cuticle of the head capsule was destroyed during the operation. When this occurred, a piece of cuticle from the hind wing of another insect was substituted and the wound was sealed in the usual way.

It was later found that an opening in the head of the adult could be made by gentle burning of the cuticle. Thus the brain was exposed and cauterisation was made.

Cauterisation of neurosecretory cells of the suboesophageal ganglion

A fine micrautery designed by Dr. C.T. Lewis was used. The pin was sharpened until its tip measured approximately 7/4. This was found satisfactory for cauterising the neurosecretory cells in the ganglion without causing any additional damage. Experiments were performed as before but the insect was placed ventrally. The head was stretched backwards and was held in position by a slight pressure of a pin. The cell was cauterised in the same way for one second only. The wound was sealed with 'Durofix'.

Controls

The insects had the cuticle of their head burnt but care was taken not to injure the brain or the ganglion. The wounds were then sealed with 'Durofix'.

4 - Farnesol technique

Farnesol was a commercial sample (95% pure) bought from L. Light and Co. It was first spread over the body of larvae, pupa and adult with the head of an entomological pin. However, it was found, in the larva, that the tufty hair covering the body clung to the pin and quite often a brush was used to separate the insect from the pin. Therefore, another simple method was used. A small camel hair brush was reduced by cutting to 2 or 3 hairs only and then used

for applying the farnesol. It was smeared twice a day with the aid of a dissecting microscope.

Rearing techniques

As the conditions in which the insects were kept varied for normal and dormant larvae, pupa and adult, the rearing techniques will be described in the appropriate sections.

f. The prothoracic glands

Mature nondormant larvae were injected with methylene blue and then they were fixed in aqueous Bouin. Dissections were made under a mixture of 50% alcohol and glycerine, using very fine pins and a high powered dissecting microscope. The best results were achieved by cutting the common tracheal trunk immediately internal to the prothoracic spiracle, releasing the fine tracheal branches from the neighbouring tissues and lifting the whole preparation onto a slide with a drop of a mixture of 50% alcohol and glycerine. Any further arrangement of structures was done on the slide. To determine whether the gland was syncytial or cellular, the preparations were examined under the phase-contrast microscope.

III ANATOMY AND HISTOLOGY OF THE CENTRAL NERVOUS SYSTEM IN LARVAE

Introduction

The life cycle of Trogoderma granarium under favourable conditions from egg hatching to the emergence of the imago lasts about 34 days for males and 40 days for females. The male larva passes through five instars whereas the female larva passes through six. Although the adult female is much heavier and larger than the male, larvae of different sex but the same instar do not differ in weight or in size. The first moult occurs after 5 - 6 days and the length of the larva at that time is about 0.9 mm. and the body width 0.3 mm. Mature larvae of both sexes are about 6 mm. long and 1.4 mm. in width.

The anatomy of the central nervous system in the larva has not previously been described and the opportunity is taken here to indicate its salient features in so far as they are relevant to this work.

Materials and technique

Larvae of the six instars were used. All were dissected, sectioned and stained as have been described in general methods and technique.

Morphology

The number and the arrangement of the ganglia are constant for all larval instars; the only difference is in the increasing size of the ganglia and the connectives. Although the mature larva develops a length about six times greater than that of the first instar, the diameters of the brain and of the ganglia increase by only twenty-five per cent. The increase in length takes place conspicuously and mainly in the connectives. (See table 1).

The nervous system (Fig. 1) consists of eleven ganglia, these being the brain, the suboesophageal ganglion, three

thoracic and six abdominal ganglia.

The brain lies immediately above the oesophagus in the head and consists of two connected pear-shaped lobes. The latter are connected anterio-ventrally and form a circumoesophageal nerve ring (Fig. 2). The oesophagus extends backward below the brain and above the suboesophageal ganglion. Anteriorly, the brain is connected with the frontal ganglion which lies above the oesophagus a short distance in front of the brain. Anterio-laterally, each lobe sends off a main antennal nerve, which runs parallel to the oesophagus. Near their anterior connection, each lobe is joined to the suboesophageal ganglion by a long para-suboesophageal connective. Ventrally and inwardly, the corpora cardiac/allata are connected to the brain by the nervi corpora cardiaci.

The suboesophageal ganglion is also pear-shaped and lies ventral to the oesophagus in the posterior region of the head. It is joined to the ventral nerve cord by two long connectives. Antero-laterally, the ganglion sends off a pair of main nerves which run parallel and ventral to the oesophagus and supply the ventral part of the head. A very find nerve arises from the maxillary nerve of the sub-oesophageal ganglion and connects the latter to the corpus cardiacum/ allatum (Fig. 2).

The three thoracic ganglia, of which the second is the largest, are almost rounded. They supply the leg muscles and musculature of the thoracic segments. They are connected to each other by long widely separated connectives, the longest in the central nervous system.

In the abdomen there are six ganglia of which the fifth is the smallest and the sixth is the largest. The first five ganglia are smaller than the thoracic ganglia and are funnel-shaped. The last abdominal ganglion is elongate and joined to the fifth by short connectives. It represents the fused ganglia of the sixth to eleventh segments.

The abdominal ganglia are displaced anteriorly so that they do not all occur in the segment of origin. The first and the second ganglia are located in the first abdominal segment, the third is in the second segment, the fourth ganglion in the third segment and the fifth and the sixth ganglia are in the fourth abdominal segment.

The nerves from each ganglion supply the segment in which it has its origin. The connectives between the abdominal ganglia are short and close together. The shortest are those which connect the fifth and the sixth ganglia.

Histology

Four distinct regions are found in the brain; an outer non-cellular neural lamella, an inner neural sheath, the cortical zone and finally the neuropile. The first two layers together form a thin connective tissue sheath which invests the brain. No structures could be differentiated in the outer neural lamella by the methods used in this study. It appears a non cellular and homogeneous layer, staining dark purple with paraldehyde fuchsin (PF) and dark blue with chrome haematoxylin/Phloxine (CHP). The inner neural sheath is composed of a continuous layer of syncytial sheath cells with very minute nuclei. It stains somewhat green with PF and red with CHP. In the first instar larva, the outer and inner sheath measure approximately 0.4 and 1.0 respectively. The measurements of the two layers in the sixth instar larva increase and become 0.8 and 1.5 respectively. The cortical zone comprises neurosecretory cells, glial cells, association and motor neurones. The neuropile, which comprises the bulk of the medullary zone, is made up principally of axons.

The suboesophageal ganglion, as the rest of the ventral ganglia, is invested by a laminated neurilemma consisting of two layers, the outer non cellular and the inner cellular neural sheath. The body of the ganglion is divided into two regions, the inner medulla or neuropile and the outer thinner cortex. The cortex is generally thicker on the ventral than on the dorsal side.

In the suboesophageal ganglion, the neurosecretory cells could be traced. Neither the thoracic nor the abdominal ganglia contain any recognisable neurosecretory cells.

In the frontal ganglion also, not a single neurosecretory cell was traced. Thus the endocrine system in the larva of T. granarium is composed of the brain, suboesophageal ganglion, corpora cardiaca, corpora allata and the pothoracic glands (Fig. 2a, b, & c).

Table I. Morphometric measurements of some ganglion and connectives in first instar and mature larvae of T. granarium based on 3 - 5 larvae of each instarr.

Part of nervous system	Mature larva	Ist-instar larva
Brain (diameters)	2I6 x I30 µ	I75 x 70 µ
Suboesophageal ganglion (diameters)	I66 x 70 µ	99 x 55 µ
2nd thoracic ganglion	I57 x 85 µ	69 x 45 µ
Ist abdominal ganglion	II0 x 53 µ	45 x 3I µ
Last abdominal ganglion	I6I x 53 µ	89 x 3I µ
Connectives between 2nd and 3rd thoracic ganglion (diameters)	238 x 22 µ	40 x 4 µ
Connectives between 3rd thoracic and Ist abdominal ganglia	I34 x I7 µ	26 x 3 µ
Number of cells in the brain	I2332	7915
Diameters of brain cells	5 x 5 µ	2 x 2 µ

IV THE NEUROSECRETORY SYSTEM IN NON DORMANT LARVAE

Introduction

Marked differences are exhibited by the instars in T. granarium in size, colour, the type and arrangement of body hairs and also in the length of the characteristic hairy brush on the last abdominal segment. Yet it was found that these characters were of limited use when it came to distinugishing one instar from another. Only the first instar could be distinguished with certainty, and each of the later instars could be recognised but there was the possibility of confusion with a successive one. Therefore, for the purpose of the present chapter, where accurate dating of different instars and stages was required, the following technique was adopted.

Rearing technique

Eggs were obtained daily from groups of adults and placed carefully in plastic petri-dishes with perforated covers to allow aeration and maintained at 35°C and 70% R.H. The base of each petri-dish was covered with black filter paper to enable the small hatching larvae to be observed. Newly hatched larvae were transferred singly to 2" x 0.5" tubes with one grain of wheat. The grains were knicked with a sterilised razor blade to make it easier for the larvae to feed on them. The larvae were examined daily for moults which were removed and new food was replaced as required. With practice, it was found that examining the tubes for the first three days after each moult could be abandoned, thus decreasing the disturbance and exposure of the larvae to low temperatures. Larvae of each instar were separately fixed always on the fourth day after each moult.

Results

The number, size, type and inclusions of the components of neurosecretory system differ from one instar to the other as indicated in Table 2.

Table 2. Numbers and types of neurosecretory cells located at different parts of the nervous system of normal larvae of different instars of T.granarium, together with volumes of C. cardiaca and allata, based on 5 larvae of each instar.

	1st Instar	2nd Instar	3rd Instar	4th Instar	Mature larva
	Brain cells - Median groups				
No. of A cells	10	14	16 - 1	20	24
cell diam.(µ)	4.5 × 5.5	6 × 7	6.5 × 7.5	8 × 9	10 × 12
nuclear diam.	2.5 × 2.5	3 × 3	3 × 3	3.5 × 3.5	4.5 × 4.5
No. of B cells	nil	nil	nil	4 - 6	6 - 8
cell diam.(µ)	-	-	-	7 × 8	8 × 10
nuclear diam	-	-	-	3.5 × 3.5	4 × 4
	Brain cells - Lateral groups				
No. of A cells	nil	nil	2 - 4	6	10 - 14
cell diam. (µ)	-	-	6.5 × 7.5	8 × 9	10 × 12
nuclear diam.	-	-	3 × 3	3.5 × 3.5	4.5 × 4.5
	Suboesophageal ganglion - mid ventral line				
No. of A cells	1	1	1	1	1
cell diam.(µ)	4 × 4.5	6 × 6	6 × 6.5	8 × 7	11 × 8
nuclear diam.	2.5 × 2.5	3 × 3	3 × 3	3.5 × 3.5	4 × 4
No. of C cells	nil	nil	nil	nil	6
cell diam.(µ)	-	-	-	-	13 × 11
nuclear diam.	-	-	-	-	4 × 4
	Volumes of Corpora cardiaca and allata				
Vol. cardiaca	39,062	51,000	63,681	74,979	89,540
Vol. allata	18,439	21,428	28,273	34,000	37,820

a - Neurosecretory cells in first instar larva

There are three groups of neurosecretory cells in the first instar larva, two median groups lying close together in the pars intercerebral region of the brain and a third group located mid-ventrally in the cortical zone of the suboesophageal ganglion.

1. In the brain (Fig. 3)

The two groups of neurosecretory cells in the brain lie deeply in the dorso-medial aspect of the protocerebrum. A group is lodged in each hemisphere and is composed of about five A cells. They stain dark purple with PF and dark blue with CHP. Each cell appears to be a unipolar pyriform neurone with a comparatively large nucleus but little cytoplasm. The latter contains minute granules or clumped secretion in masses of varying sizes but never exceeding 0.5μ. Although the granules are found scattered in all parts of the perikaryon, they are densest in the peripheral regions of the cell. The rest of the cytoplasm stains pale pink and contains minute vacuoles. Granules of neurosecretion can also be traced along the axons of the cells. The nucleus lies in the centre of the cell and contains considerable amounts of chromatin.

2. In the suboesophageal ganglion (Fig. 4a)

The suboesophageal ganglion contains the third group of the neurosecretory cells which is represented by one A cell comparable to those of the median groups in the brain. The cell lies deeply in the mid-ventral line of the ganglion. Granules are foundin the cell and can also be traced along the axon. The cell measurements are similar to those of the brain cells.

b - Neurosecretory cells in second-instar larva

Three groups of neurosecretory cells are found in the brain and the suboesophageal ganglion of the second instar larva, their location and arrangement being exactly the same as in the first instar larva.

1. In the brain (Fig. 5)

The secretory neurones of the median group of this instar contain more inclusions and fewer vacuoles than the cells in the first instar larva, but the granules are of the same size and they are densest in the peripheral parts and in the vicinity of the vacuoles. There is relatively more cytoplasm than in the first instar and the nucleus has increased slightly, containing more chromatin which stains more deeply in the haematoxylin used in PF and CHP.

2. In the suboesophageal ganglion (Fig 4b)

The suboesophageal A cell has the same location of that in the suboesophageal ganglion of the first instar. The cell inclusions are similar to those of the brain cells.

c -Neurosecretory cells in the third instar larva

In these larvae, five main groups of neurosecretory cells are found in the brain and the suboesophageal ganglion. Two groups of these cells, the median groups, lie in an antero-dorsal position in the pars-intercerebralis region close to the junction of the hemispheres. Two other groups of cells, one in each cerebral hemisphere, lie dorso-laterally to the median groups in the vicinity of the origin of the optic and antennal nerves and constitute the lateral groups. The fifth group is located in the suboesophageal ganglion.

1. In the brain (Fig. 6)

The two median groups are now represented by 16 - 18 secretory neurones, eight to nine in each hemisphere. Each group occupies an area about 3,000 μ^2 and is covered by a layer or two of vacuolated cells beneath the brain membrane. All are A cells. The cells contain minute particles which associate to form aggregates or granules. Although these granules nearly remain limited in size as in the previous instar, they increase in abundance and are densest in the sub-spherical zones around the vacuoles which are now rather small.

The lateral groups, lying in the vicinity of the

of the origin of the antennal nerves, consist of 2 - 4 neurones, 1 - 2 in each hemisphere. Each cell is approximately spherical and has more or less the same structure of that in the neurosecretory cells of the median groups but is slightly smaller.

2. In the suboesophageal ganglion (Fig. 4c)

In the suboesophageal ganglion, not more than one cell could be traced. It lies deeply in the mid-ventral line in the cortical zone of the ganglion covered by a layer of non secretory cells beneath the ganglion membrane. The cell is unipolar, pyriform and stains deep purple with PF. it is thus an A cell. The granules of neurosecretory material are rather small, filling the perikaryon of the cell except for a few vacuoles. The general facies resembles that of a brain cell belonging to the median group. Neurosecretory material can be traced also along the axon hillock.

d - Neurosecretory cells in fourth instar larva

There are also five groups of secretory neurones, two median groups, two lateral groups in the brain and one group in the suboesophageal ganglion.

1. In the brain (Fig. 7)

Each median group is lodged in the dorso-medial aspect of the pars intercerebralis of the protocerebrum of each hemisphere of the brain. The group is about 50 μ wide and 60 μ long and extends about 16 μ into the brain near the mid-line. It consists of ten A-cells and 2 - 3 B cells. The two lateral groups are represented by six neurosecretory cells, three in each lobe, lying lateral to the median group. They are all A cells.

Compared with earlier instars, the amount of product in the cells has increased remarkably and the deep purple granules in PF stained specimens become larger. Vacuoles decrease and the nucleus becomes rich in chromatin. The A cells of the median group are pyriform; they stain very dark purple and the aggregates are conspicuous, whereas the cells of the lateral group are sub-spherical, stain lightly and the granules are conspicuously smaller.

2. In the suboesophageal ganglion (Fig. 4d)

The fifth group lies in the ventral mid-line of the suboesophageal ganglion. It consists of one A cell lying deeply near to the medullary zone. The cell is comparable to those of the median groups in the brain. The secretory products are being transmitted along the axon in much greater amount than the previous instars and the axons can easily be traced through the medullary zone.

e - Neurosecretory cells in mature larvae

The arrangement and the number of the neurosecretory cells in the brain and the suboesophageal ganglion of both fifth and sixth larval instar are similar. The only difference between these larvae lies in the size of the neurosecretory cells, which are smaller in the fifth instar.

1. In the brain (Fig. 8)

Four groups of neurosecretory cells are found in the brain, two median groups and two lateral groups. The median groups lie in the usual region as in the previous instars. They lie deeply, close to the junction of the two hemispheres. In some specimens, the two groups appear at first sight to form a single cluster but their separate identities can be established by the clear pathways of their axons. In each group, there are 12 A cells staining deep purple with PF and blue black with CHP and 6 - 8 B cells which stain bluish green with the light green component of PF and are strongly phloxinophilic when stained with CHP.

The A cells are the most conspicuous. They often appear pear-shaped on both transverse and sagittal sections with a prominent axon leaving the cell at the apex of the narrow end. The dimensions of these cells can show some variations from one sectioned individual to another. At low magnification, they appear to have a uniform deep purple colour broken only by the nucleus. At high magnifications, this colour is resolved into minute purple particles. These small particles appear to associate to form aggregates or granules of varying magnitude filling most of the cell bodies and their axons. The maximum size of

the granules is 0.5u. These granules give the cell type its characteristic tinctorial affinities. Where the cells are large, the inclusions are also large, broken by very few vacuoles.

The B - cells are almost spherical neurones and are situated among the A - cells. In some preparations, the green component of the PF does not take and the B - cells do not show their special tinctorial affinity. With CHP, they stain red and they contain phloxinophil inclusions of minute size, never as large as the inclusions within the A - cells.

The two lateral groups, one in each cerebral hemisphere, lie dorso-laterally to the median groups in the vicinity of the origin of the optic antennal nerves. Each group consists of five to seven cells. All are of the A type. They are spherical cells and their staining affinity is similar to A cells of the median group using PF, CHP and azan; but the cytoplasmic inclusions are slightly different. The granules are not as big as those of the median group and the whole cell is smaller, though the nucleus attains the same size.

2. In the suboesophageal ganglion (Fig. 4, e and 9)

In the suboesophageal ganglion, there is, as usual, one A cell, comparable to those of the lateral group in the brain. It lies deeply in the mid-ventral line in the cortical zone of the ganglion. The cell is unipolar, pyriform and the product can be traced along the axons which run right across the whole width of the medullary zone.

Apart from this A cell, there are five large cells with comparatively large nuclei, lying intimately together in the mid-ventral line of the ganglia but deeper than the A cell. They stain reddish with PF and blue cytoplasm with red inclusion; thus they are C cells. They are bigger than the A cells of the median group and contain bigger nuclei, but smaller aggregates. They have extremely prominent axons which run deeply in the medullary zone of the ganglion. The granules in the cells are scantily scattered through the cytoplasm.

Neurosecretory pathways

The pathways traversed by the axons of the A cells and B cells in T. granarium are typically the same as in all insects so far studied (Van der Kloot, 1960). They are very distinct owing to the presence of stained secretory material carried along them. The most conspicuous pathways are the ones which are traversed by the axons of the A cells. The pathways traversed by the B cells are, however, less defined. Their secretions are probably less in amount and light in colour, therefore are masked by the A cell secretions. Because their axons run along those of the A cells initially, it is possible that they continue their entire pathways together. Studying various serial sections, it is found that the axons from the neurosecretory cells of the two median groups pass anteriorly into the neuropile. Then they curve posteriorly and decussate, entering the internal corpora cardiaci nervi (NCCI) Thus each corpus cardiacum is innervated by axons from the opposing medial group of neurosecretory cells.

The axons from the lateral group of neurosecretory cells run directly without decussating and enter the external corpora cardiaca nervi (NCCII). Their pathways, however, are never as distinct as those of the medial group of cells. The NCCII can be traced easily from the ventral surface of the brain to its connection with the NCCI. The latter is a relatively large nerve whereas NCCII is a very fine one.

The two nerves NCCI and NCCII become attached together posteriorly soon after they leave the brain. They then communicate with the corresponding internal part of the corpora cardiaca. These two pairs of nerves innervating the corpora cardiaca occur in all pterygote insects (Cazal, 1948).

The neurosecretory pathways of the suboesophageal ganglion can only be traced up to the dorsal margin of the medullary zone, beyond which their direction is unknown. In the larva of the sixth instar, the axons of the five peculiar cells and of the A cell continue their entire pathways together. It is reasonable to assume that the neurosecretory products of the ganglion are discharged along the nerves arising from the ganglion as there is no other way for the release of the secretion. This assumption agrees with the findings of Delphin (1963) in Schistocerca gregaria.

f - The corpora cardiaca (Figs. 10 - 15)

Each corpus cardiacum is an elongated structure and situated very close to the brain. It is connected with the brain by the two nerves NCCI and NCCII. The two corpora cardiaca are directed laterally and downwards and intimately associated with the walls of the aorta. They are, therefore, considered to be 'Neuro-haemal' organs in the sense of Carlisle and Knowles (1953).

The corpus cardiacum is of nervous origin (Hanstrom, 1940) but neurones are uncommon in the functional origin. Histologically, the corpus cardiacum of T. granarium consists of two types of cells embedded in the matrix; but it does not show a differentiation into an anterior and a posterior region as has been detected in most insects (Cazal, 1948). Two types of nuclei are seen. The larger nuclei, which appear to be secretory owing to the presence of a secretory product surrounding them; and smaller nuclei which appear to be relatively inactive. The first type of nucleus is found mainly in the peripheral parts of the gland, thus forming an ill-defined cortex. They stain dark purple or brownish purple with PF. These cells correspond to the 'chromophile cell' described by Cazal (1948).

The second cell type appear to be non-secretory and are scattered in the matrix of the corpus cardiacum. Few of these cells can be traced among the secretory cells of the periphery. They are probably comparable to the 'chromophobe' cells of Cazal (1948).

The axons from the NCCI and NCCII intermingle in the anterior portion of the corpus cardiacum, which has taken the functional role of storage of the neurosecretory materials from the brain. Droplets of stainable secretory material lie within or outside the axons between the chromophile and chromophobe cells. Some secretory granules have been observed intracellularly suggesting that there is probably an intimate relationship between the axons of the neurosecretory cells and the secretory cells of the corpus cardiacum. This problem may be solved by electron-microscopy. The size of corpora cardiaca in all larval instars is shown in Table 2, page 18.

g - The Corpora allata (Figs. 10 - 15)

They are roughly spherical and they are attached to the lower extremity of the respective corpora cardiaca. Each corpus allatum is invested by a thin connective membrane which is continuous with that of the corpus cardiacum. The gland consists of packed cells which are distributed homogeneously. All cells are of one type. The cell outlines are either clearly seen or discerned with difficulty according to the different levels of activity of the individual cells. The cells are spherical or subspherical with spherical nuclei. They stain bluish green with the light green component of PF. Vacuoles can be discerned or are wanting according to the activity of the gland. Very few globules of neurosecretion from the brain have been traced in the gland. There is also a substance which stains strongly with paraldehyde-fuchsin and shows densely in the peripheral parts of the gland. This substance consists of larger globules than those from the brain and it is associated with the tracheae of the gland. This should not be interpreted as equivalent to the brain secretory materials. According to Fraser (1959) who has made the same observations in Lucilia, about to moult, this substance is the tracheal chitin which has been attacked by the moulting fluid, and the chitin precursor in the tracks of the new tracheae.

Some of the axons of NCCI and NCCII which innervate the corpus cardiacum traverse their entire length into the corpus allatum. These axons may be considered to constitute the cardiaco-allatum nerve which is very distinct in some insects (Cazal, 1948). However defined, nervi allati do not exist in T. granarium. Sizes of corpora allata in all larval instars are given in Table 2 on page 18.

h - The prothoracic glands (Fig. 2c)

The prothoracic are comparatively large. The glands are situated in the cervical region closely applied to the dorsal tracheal trunk, and are attached to the origins of the ventral trunk. The glands extend posteriorly in the prothoracic cavity forming a sheath over the dorsal tracheal trunk till its connection with the ventral trunk. Unlike Anthrenus vorax (Dermestidae) described by Srivastava (1959) the glands are not syncytial, but are made of cells with comparatively large nuclei. The cells measure approximately 8 x 6u and the nuclei measure about 6.5 x 4 u. No nerve supply could be traced.

V THE NEUROSECRETORY SYSTEM IN DORMANT LARVAE

Introduction

Arrested growth, diapause or quiescence, is now appreciated as a highly complex mechanism for the preservation of the species in regions where seasonal climatic conditions are unfavourable for continuous growth and for reproduction. In Arthropods with a facultative diapause, the arrest of growth is ultimately governed by the environment. In such species, the capacity for diapause may be realised in any generation or may remain latent indefinitely, according to the external conditions.

These external factors influence the physiological and biochemical control of growth. It is now well known from the classical researches of Wigglesworth, Fukuda and Williams that the cessation of growth and the accompanying metabolic adjustments are under hormonal control. The neurosecretory system is thought to provide the link coordinating the internal physiology of the insect with the external environment. Indeed, it is this link, as Lees (1955) suggests, that permits the mechanism under-lying diapause to function as a timing device synchronising the periods of dormancy and active growth with the rhythm of the environment in general.

It has been known for many years that insects suffer an arrest of growth when deprived of the source of the moulting hormone. As early as 1917, 1922, Kopec showed that the larva of Lymantria is prevented from pupating if the brain is removed and suggested that the brain was the source of the hormone necessary for moulting. He found that caterpillars deprived of the brain could survive for many weeks as 'Dauer-larvae'. Wigglesworth (1934) suggested that diapause in insects may be due to a temporary failure in the secretion of a growth hormone. Since then, a vast amount of evidence has proved that the brain plays a controlling role in many other species with a larval or pupal diapause, e.g., Ephestia (Kuhn and Piepho, 1936), Deilephila (Plagge, 1938), Trypoxylon, (Schmieder, 1942), Platysamia and Telea (Williams, 1942,

1946) and Gryllus campestris (Sellier, 1949).

It was not until 1940 that Wigglesworth provided the evidence that it is not the whole brain that is responsible for moulting and growth but it is only the neurosecretory cells in the pars intercerebralis that are responsible for metamorphosis. Williams (1952b) confirmed these results in Platysamia.

As far back as 1931, Hachow found that the pupal development of Vanessa and Aparia is initiated by some centre located in the thorax. These results were confirmed in Phryganidia by Bodenstein (1938) In 1940, Fukuda identified the thoracic centre with the 'prothoracic glands', and he discovered their functional role, their control of moulting and development of the larva and pupa of the silkworm Bombyx mori by ligaturing experiments. The relation between the brain and the thoracic glands in H. cecropia was established experimentally by Williams (1947). When he implanted an activated brain, i.e., one which had been chilled previously, into the isolated abdomen of a diapausing pupa, it did not induce development, whereas it did cause metamorphosis of the anterior half from which the brain had been removed. However, an isolated diapausing abdomen could be induced to metamorphose if it was provided with an active brain plus prothoracic glands. Williams subsequently (1948b, 1952) established that the source of the activation of the prothoracic glands came from the neurosecretory cells of the brain. Thus in H. cercropia, pupal diapause is due to the inactivity of the neurosecretory cells of the brain and the prothoracic glands. Chilling activates the brain which in turn activates the prothoracic glands

This work has been confirmed in other families and orders by several workers. They concluded that the thoracic glands give rise to the hormone that induces growth and moulting. To do so, however, the prothoracic gland cells have to be activated by the secretion from the neurosecretory cells of the brain.

The corpus allatum plays a great role in controlling metamorphosis. The gland was shown experimentally to be responsible for secreting the Juvenile hormone in many insects of different orders: e.g., Hemiptera (Wigglesworth, 1934, 1936), Orthoptera (Pflugfelder, 1937), (Scharrer, 1946a), Lepidoptera (Piepho, 1938a, b), Diptera (Bodenstein, 1939b), Coleoptera (Radtke, 1942) and Neuroptera (Rahm, 1952).

This hormone inhibits metamorphosis and causes the retention of the larval characters.

The evidence reviewed in the foregoing pages shows that the inception of diapause and arrest of metamorphosis are associated with the temporary failure of some component of the endocrine system, mainly the neurosecretory cells of the brain.

In addition, some investigators of diapause found that the suboesophageal ganglion is responsible for secreting a diapause hormone; e.g., Fukuda (1951a, b, 1952 and 1953 a, b) and Hasegawa (1952). The liberation of the hormone, however, depends in turn upon the brain which either restrains the liberation of the hormone or causes its release. This influence is exerted through circumoesophageal commissures.

The activity of the brain and suboesophageal ganglia, however, has been found to be determined by the internal and external environments. Some authors have found that high temperature activates the brain (e.g., Way and Hopkins (1950) in Diataraxia and Lees (1953a) in Metatetranychus) and activates the suboesophageal ganglion in Periplaneta americana (Harker, 1960). Others have found that low temperature stimulates the brain, e.g., Williams (1947, 1952b) in Platysamia and Highnam (1958) in Mimas tilia.

The mode of action of temperature and food has not been fully resolved. Titschack (1926) caused Tineola biselliella to cast their skins as many as eight times in 3 days if deprived of food. In 1931, Kemper considered that the distention of the abdomen by a blood meal was an important factor in the initiation of growth and moulting in Cimex lecturarius. It was not until 1934 that Wigglesworth proved that stretching of the abdomen of Rhodnius provides the nervous stimulus to the brain that brings about the necessary activity in the neurosecretory cells. Clark and Langely (1963) suggested that the relaxation and distention of the gut that occurs during ecdysis provides the stimulus for the release of the growth and moulting hormone in Locusta migratoria. According to Simmonds (1948) and Andrewartha (1952) temperature and food influence the activity of the brain indirectly through the medium of metabolism. On the other hand, Lees (1955) suggests that temperature should be regarded as a 'token' stimulus directly on the brain and it may also react with the metabolism of insects. Harker (1960) found that

temperature has a direct effect on the activity of the suboesophageal ganglion of Periplaneta.

Trogoderma granarium is a persistent pest whose dormancy is facultative and sometimes lasts for more than four years (Burges, 1959). Food and temperature play the major part in the induction and termination of dormancy. The work of Burges (1959, 1960 and 1962) represents a detailed study of the biology of diapause larvae under favourable and unfavourable conditions.

The role of the neurosecretory system in the control of induction and termination of dormancy in Trogoderma in response to the effects of the external environment will now be considered together with the endocrine-metamorphosis relationship.

Materials and techniques

Diapausing larvae were supplied by Dr. Burges from the Pest Infestation Laboratory, Slough. They were fourth and mainly fifth and sixth instar larvae and were two weeks advanced in dormancy. The larvae were kept in a glass jar and fed with whole grains of wheat. A suitable 'refuge' for the larvae was constructed, comprising two rectangular pieces of cardboard lined with cotton fabric and separated along the one side by a narrow strip of the same cardboard, the whole structure being held together by elastic bands at each end. This 'refuge' provided a narrow crevice of triangular cross-section, for larvae of all sizes. The culture was raised at 25°C. and 65% R.H. The larvae were divided into four groups, three of which were transferred to other jars as follows:

1. Jar No. 1 contained about 50 dormant larvae. Food and refuge were not supplied and the culture was raised at 25°C. and 65% R.H.
2. Jar No. 2 contained 50 dormant larvae and was raised at 30°C. and 65% R.H. The larvae were supplied with the same kind of food as in the stock cultures. No refuge was provided.
3. Jar No. 3 contained about 50 dormant larvae and was also raised at 30°C. and 65% R.H. but neither food nor refuge were supplied.

The rest of the dormant larvae, which numbered about 150 larvae, were left in the original jar, No. 4 with food and refuge and

were kept at 25°C. and 65% R.H. Thus larvae were compared at two temperatures (25° and 30°C.) with and without food.

As dormant larvae may feed, moult and pupate, all jars were examined daily and the cast skins and pupae were removed.

Samples of larvae from the four jars were separately fixed at one week intervals.

For analysing the varying amounts of stainable material in the neurosecretory system at different stages, the neurosecretory cells were divided into eight arbitrary categories designated as stages I to VIII. A cell in stage I had little neurosecretory material, whereas cells in stage VIII were extremely packed with aggregates of neurosecretion.

Relative quantities of neurosecretory cells' secretion, discharged along the axons at the time of fixation, were represented by arithmetical symbols from _, ±, +, ++ and +++.

Relative quantities of different secretions of neurosecretory cells stored in the corpora cardiaca at various stages were represented by the same symbols as above.

The mode of action of temperature on neurosecretory system

To investigate whether temperature influences the discharge of the neurosecretory cells rapidly and directly, the following method was devised.

Mature non-dormant and dormant larvae were cut behind the neck under Ringer's solution at two different temperatures, 10° and 50°C. They were kept at these temperatures for a period of ten minutes and then fixed with aqueous Bouin of the same temperature.

Results

The histological and morphometric changes of the neurosecretory system in dormant larvae kept in the four different environments are summarised in Table 3. page 32.

TABLE 3. The histological and morphometric changes in dormant larvae maintained in different enviroments; based on 6 - 10 specimens each state.

Environments	Age in weeks	Neurosecretion in cell body			A-cell secretion along the axons	Storage in C. cardiacum		Cell volume in u^3			Nuclear volume in u^3			Volume of C. cardiacum u^3	Volume of c. allatum u^3
		A	B	C		A	B	A	B	C	A	B	C		
25°C with food	2	IV	III	III	+	++	?	320	297	390	29	27	33	81230	36100
	4	V	III	IV	±	++	?	312	280	380	28	26	31	79880	31100
	6	VI	IV	V	±	+	+	290	250	380	26	24	30	68899	29890
	8	VII	V	V	±	++	+	235	245	332	21	24	28	81000	28290
	10	VIII	V	VI	-	+++	+	230	210	310	-	20	26	79260	28820
25°C without food	2	III	III	III	±	+	±	310	290	318	26	24	30	71000	30000
	4	II	II	III	±	+	+	240	210	300	22	20	27	69810	29900
	6	II	II	II	-	±	±	130	189	298	14	18	26	62000	25000
30°C. with food	1	III	II	II	++	+	±	380	300	395	34	29	33	79200	32000
	2	II	II	II	+++	±	±	410	312	480	42	31	34	82000	34900
	3	II	II	II	+++	±	?	470	314	556	47	34	38	87500	36600
	1	I	I	I	±	±	±	218	210	310	21	20	28	68820	36300
	2	I	I	I	-	-	-	185	190	290	19	18	27	59000	29800
	3	I	I	I	-	-	-	121	162	280	18	16	27	51000	18000

A. Neurosecretory system in dormant larvae kept at 25°C and 65% R.H. with food

1. Neurosecretory cells in the brain

The number of A - cells in the brain remains the same as in nondormant larvae, i.e., twelve A - cells in the pars intercerebral region (median group) and 5 - 7 A - cells in the lateral group. However, the histological structure of the cells differs from that of the nondormant larvae. In two weeks dormant larva, the deep purple product, stained with PF, occurs in larger granules which associate in large irregular masses separated by relatively clear zones of cytoplasm. The vacuoles decrease and few axons hold secretory products (Fig. 16, stage IV and Fig. 17). During the next two weeks, aggregates continue to increase in size filling most of the perikaryon and the vacuoles become progressively smaller. The secretory product increases in the axon hillocks and very little secretory product can be traced in the pathways (Fig. 16, stage V). The accumulation of the granules continues and the staining reactions of the neurosecretory cells become very intense (Fig. 16, stages VI, VII and Fig. 18). By the end of the tenth week the product is so dense that it entirely fills the cells and the vacuoles completely disappear. At the same time, the cells become smaller, lose their specific pear-shape and the nucleus becomes pycnotic (Fig. 16, VIII and Fig. 19). The product is pushed densely along the axon hillocks which become very conspicuous but products in the axons are never seen.

This is the pattern that the majority, 80% - 90%, of the A - cells take during a ten week dormancy. However, a few dormant larvae show signs of secretory activity. The cells contain little product and the cytoplasm takes up stain rather poorly. The axons are full of secretions which are carried along the pathways. These larvae are, presumably, breaking diapause or moulting. Highnam (1958) observed the same signs of activity in diapausing Mimas tilia when subjected to a low temperature for periods of less than four weeks. He thought that those individuals are breaking diapause prematurely when subjected to a low temperature.

The B - cells are the most remarkable feature of dormancy in T. granarium. Although it was difficult to trace the B - cells in the non-dormant larvae, and in some of them the cells were completely unidentified, none of the specimens of dormant larvae failed to show the B - cells. The number varied from 5 - 7 in the 'median group'; none was found in the lateral group. They are spherical and full of product. While this product in nondormant larvae is in the form of minute particles, in dormant larvae, the product becomes remarkably associated forming large granules. Vacuoles are very few and axons are discernible only with difficulty. The pathways of the B - cell secretion in T. granarium as in many other insects previously studied, are usually hard to identify.

The quantity of A - cell secretion, found in the corpus cardiacum, fluctuated during the ten weeks of dormancy, depending presumably on the amount of secretion transported along the axons and the activity of the gland. A few of the larvae which had remained dormant for ten weeks, showed a large amount of the A - cell secretion in the corpus cardiacum, whilst some larvae showed very little of A - cell secretion in the gland.

The B - cells secretion stored in the corpus cardiacum, may have followed a similar pattern depending on the activity of the B - cells. However, this is not certain because the amount of B - cell secretion is usually doubtful as the secretion from the 'chromophile cells' of the corpus cardiacum has the same tinctorial affinity as the B - cells secretion.

2. Neurosecretory cells in the suboesophageal ganglion

As in non-dormant larvae, the suboesophageal ganglion contained only one A - cell. During the ten weeks of the dormancy, it passed through the same stages as that of the brain cells except the pyonotic stage. The cell and its nucleus decreased in volume, being smallest at the end of the ten weeks of dormancy. The axons disappear completely.

The C - cells, whenever present, were full of secretion, their size decreased and their axons were indiscernible (Fig. 20).

3. The corpora cardiaca (Fig. 21)

The values found for the volume of a corpus cardiacum fluctuated throughout the ten weeks of dormancy. However, the gland in dormant larvae was smaller than in non-dormant larvae. The secretory 'chromophile cells' shrank correspondingly with the size of the gland.

4. The corpora allata (Fig. 21)

There was also fluctuation in the values found for the size of the corpus allatum, butan initial small decrease in its volume was observed compared with that in non-dormant larvae. After ten weeks of dormancy, the gland attained the smallest volume recorded. The cells of the gland correspondingly decreased in size and became closely packed. Their cytoplasmic content became low, their nuclei shrank and the vacuoles disappeared.

B. Neurosecretory system in dormant larvae kept at 25°C, 65% R.H. and without food

The larvae moved actively during the first week then the movements almost ceased. Very few cast skins were found compared with those which were cast by larvae supplied with food. About 20% of the larvae pupated and 8% died within six weeks.

The qualitative and quantitative changes in neurosecretory system of larvae maintained in this environment are shown in Table 3. on page 32.

1. Neurosecretory cells in the brain

Compared with previous treatment the results suggest that whereas in larvae at 25°C with food neurosecretory substance was formed but discharged very slowly, when food was not present at 25°C neurosecretory substance was apparently not formed or formed only slowly.

The brain A - cells passed through gradual changes during

the six weeks dormancy. In larvae which had remained in dormancy for two weeks the A - cells contained irregular masses of product separated by clear zones of cytoplasm and few vacuoles (Fig. 16, stage III). By the end of the fourth week the aggregates decreased in number and abundance and the product occurred in small granules. The cell also showed a slight decrease in size and this was more conspicuous in the cytoplasmic region than in the nucleus (Fig. 16, stage II). In larvae which had remained six weeks in dormancy the cells became spherical and there was a conspicuous decrease in the cell size, a diminution in the amount of the product in the cell and the axons completely disappeared (Fig. 16, stage II and Fig. 22).

The number of the B - cells varied from 2 - 5 during the six weeks. In some specimens the B - cells were poorly stained and in others, the majority, were clearly seen. The changes in the B - cells were no less spectacular than those in the A-cells.

During the first two weeks the product consisted of small aggregates scattered in all parts of the perikaryon (stage III). During the other four weeks the product decreased and the cells depleted of secretion (stage II).

The amount of A - cell secretion transported along the axons was very little in larvae which had been two weeks in dormancy. By the end of the sixth week the secretion was wanting and the pathways became difficult to trace.

Very little of A and B secretions were observed stored in the corpus cardiacum.

2. Neurosecretory cells in the suboesophageal ganglion

The changes of neurosecretory cells in the suboesophageal ganglion during the six weeks of dormancy are no less spectacular as compared to the brain cells. The A - cell passed through stage III and stage II during dormancy. The axons were undiscernible. The C - cells contained very little secretion and their size decreased (Fig. 23).

3. The corpora cardiaca

The volume of the corpus cardiacum gradually decreased.

By the end of the sixth week of dormancy the gland, compared with the previous treatment, was more shrunken. The secretory 'chromophile cells' which stained green with PF shrank correspondingly with the size of the gland and there was also a decrease in the nuclear volumes (Fig. 23).

4. The corpora allata.

There was a conspicuous initial decrease in volume of the gland during the first two weeks of dormancy. This small volume was maintained throughout the whole period. The cell-boundaries were ill-defined and the nuclei were closely packed and homogeneously distributed in the matrix of the gland. Vacuoles were undiscernible (Fig. 23).

C. Neurosecretory system in larvae kept at 30°C, 65% R.H. and supplied with food (Table 3).

By the end of the third week all the larvae had pupated. Many pupated during the first two weeks. Histologically, all the specimens which had been examined showed signs of secretory activity.

1. Neurosecretory cells in the brain

The brain A - cells contained a little secretion, in the form of small aggregates of varying magnitude. Vacuoles were present and relatively large. The cells regain the same appearance of the A - cells found in non-dormant larvae.

B - cells decreased in number. Not more than 2 - 3 cells could be found in any specimen that was examined. The general appearance of the cells resembled that of the B - cells in non-dormant larvae.

The amounts of A secretion transported along the axons increased remarkably (+++) and the pathways became rather clear. All the specimens which were examined showed that the amount of A - cell secretion stored in the corpus cardiacum was very small ($\pm$).

The B - cells secretion transported along the axons was less easily identified. It was only discernible till the axons entered the neuropile. The amount of B secretion stored in the corpus cardiacum varied approximately with the appearance of the B - cells and the amount

of inclusions the latter contained. However, it was difficult to ascertain the amount of the B - cell secretion found in the gland as the secretion of the 'chromophile cells' of the latter has the same tinctorial affinity as the B - cell secretion.

2. Neurosecretory cells in the suboesophageal ganglion

The A - cell resembled that of the ganglion in non-dormant larvae. The amount of secretion in the cell showed conspicuous fluctuations. Vacuoles were present and the nucleus was relatively large. The axons were conspicuous, thus denoting greater secretory activity.

Unfortunately, as always in non-dormant larvae, it has not been possible to trace the termination of these axonal pathways.

The five C - cells regain the same size, histological structure and apparent activity as the C - cells found in non-dormant larvae.

3. The corpora cardiaca

The gland attained the same size of that in non-dormant larvae. The secretory 'chromophile cells' enlarged correspondingly with the size of the gland, accompanied by an increase in their nuclear volumes. Vacuoles were relatively large.

4. The corpora allata

The sequence of changes in the corpus allatum was no less spectacular than that in the corpus cardiacum. The gland regained the volume exhibited by non-dormant larvae. This was accompanied by a marked increase in the cytoplasmic content of the cells, increasingly well defined cell boundaries, a heterogeneous distribution of the cells and the occurrence of vacuoles.

D. Neurosecretory system in dormant larvae kept at 30°C, 65% R.H. and without food

A very small number of larvae broke dormancy and pupated

during the first week. The rest gradually decreased in size till they resembled 'morbid' larvae. About 20% died within four weeks. The striking feature about these larvae is that they cast skins several times.

1. Neurosecretory cells in the brain

The A -cells and their nuclei decreased in size. The cells were depleted, containing only very little purple particles, stained with PF, appeared just detectable in the sub-spherical zones (Fig. 16, stage I and Fig. 24). Axons were almost obscure.

B - cells were difficult to identify. In a few specimens 1 - 3 cells could be found; they contained a very small amount of secretion and they were poorly stained.

The amount of secretion transported along the axons was quite small during the first week. Then the amount decreased until, now and then, only a few traces of secretion were seen along the pathways.

No evidence of secretion stored in the corpus cardiacum was found.

2. Neurosecretory cells in the suboesophageal ganglion

The A - cell was depleted of secretion and the axon could not be seen suggesting that the synthesis and discharge of neurosecretion were at a very low rate.

The C - cells were seen in very few specimens and they followed the same pattern as the A - cell.

3. The corpora cardiaca

The gland was conspicuously shrunken. The gland cells were ill-defined. Neurosecretory material from the brain was indiscernible (Fig. 25).

4. The corpora allata

The corpus allatum was also reduced in size. The gland cells' boundaries were indiscernible and the general appearance of the gland was that of a syncytial body (Fig. 24).

E. The effect of temperature on the release of secretory products

The neurosecretory cells in the larvae kept in Ringer's solution at 10°C, contained a large amount of secretion, whereas in larvae dissected in Ringer's solution at 50°C, the neurosecretory cells contained only a little secretion in the form of minute aggregates.

Although it is possible that some leakage has occurred, this simple preliminary experiment indicates that temperature has a direct effect on the release of the secretory product by the neurosecretory cells. At the higher temperature, the rate of release was greater. The axons of the neurosecretory cells held at 50°C for 10 minutes contained more secretory material than the corresponding axons ofsimilar larvae held at 10°C or at the normal rearing temperature of 30°C.

Discussion

The evidence presented indicates that dormancy of T. granarium at least in the strain provided by Dr. Burges is more likely to be a quiescence than a real diapause. It has been shown in the present work that when dormant larvae were exposed to the favourable environments which are, in this case, high temperature and fresh food, all soon broke dormancy and pupated. Some of the dormant larvae broke dormancy after two weeks. These were, presumably, dormant fourth-instar larvae. Burges (1959) maintained that dormancy of some larvae of T. granarium is considered as a quiescence whereas of others it is a real but weak diapause.

From the foregoing results evidences indicate that the activity of the neurosecretory system in T. granarium is determined by the external environment. This is a general condition in all insects. Food and temperature play a great role in the synthesis and release of

hormones of the neurosecretory system of T. granarium. In the presence of food and low temperature the neurosecretory cells are filled with 'hormone' but release of secretion is at a low rate. Absence of food and low temperature result in little hormone in the cells and a low rate of release. In the presence of high temperature and food the synthesis and release of hormone would go concurrently, i.e., the cells become active synthetically and exhibit a high rate of release. In comparison, the absence of food at high temperature is correlated with the presence of little hormone in the cells and consequently a low rate of release. It would appear, therefore, that the roles of food and temperature in governing neurosecretion can be separated to an appreciable extent.

In T. granarium food may stimulate the liberation of the brain in two ways, first by providing pre ursors for the synthesis of hormones. In the presence of food, the cells are able to build up stores of hormone, i.e., the synthesis of hormone may be directly dependent on the availability of metabolites to the neurosecretory cells, for though a temperature of 30°C permits synthesis, in the absence of food none is formed. This is perhaps a form of control mechanism whereby the insect is prevented from pupating before the necessary food reserves have been established (see Wigglesworth, 1963b). The second way is probably via nervous stimuli. Thus for example, the work of Wigglesworth (1934) showed that in Rhodnius, it is the degree of distention of the abdomen which provides the stimulus for liberation of the moulting hormone; and that section of the nerve cord in the prothorax interrupted the conduction of this stimulus to the brain. Van der Kloot (1960, 1961) also demonstrated the existence of stretch receptors in the abdomen of Rhodnius which lead to the appearance of impulses in the nerves to the corpus cardiacum. He maintained that these receptors continue to discharge as long as the abdomen is expanded. In 1963, Clarke and Langley suggested that movement of the Pharynx activating the stretch receptors stimulates nerves to the frontal ganglion which in turn maintains continuous secretion of neurosecretory material from the brain of Locusta migratoria.

On the other hand, it has also been shown in the present work that some starving dormant larvae, raised at 30°C, cast skins several times. Possibly, the explanation for this is that the larvae,

presumably, swallow air and in consequence the pharynx moves and the abdomen expands; thus necessary activity in the secretory cells is exhibited and moult occurs. Absolute starvation caused a precocious pupal formation in Galleria mellonella (Metalnikov, 1908) although merely underfeeding or intermittent starvation will delay pupal formation (Kopec, 1922). Tineola biselliella cast their skins as many as eight times in three days when they were deprived of food (Titschack, 1926).

In T. granarium the temperature by its effect on metabolism presumably affects the rate of hormone synthesis but its most obvious effect is on the rate of release of the hormone. The higher the temperature, the faster the release of hormone. Low temperatures appear to depress the release of the brain hormone from the neurosecretory cells; thus growth and metamorphosis are retarded. Similarly, high temperatures induce the brain to release its hormone in Platysamia (Williams, 1946, 1947, 1948, 1949), in Diataraxia oleracea (Way and Hopkins, 1950) and in Rhodnius (Wigglesworth, 1952b). In Platysamia, however, previous chilling of the brain for at least 6 weeks is necessary. In contrast, the brain factor is released rapidly at low temperature in Mimas tiliae (Highnam, 1958) and Bupalus piniarius (Schoonhoven, 1962).

A question arises: Do temperatures and food influence the activity of the brain directly by acting as a stimulus or through the medium of metabolism? According to Simmonds (1948) and Andrewartha (1952), temperature and food influence the activity of the brain indirectly through the medium of metabolism. On the other hand, Lees (1955) suggests that temperature should be regarded as a 'token' stimulus acting on the brain but it may also react with the metabolism of insects. The present work suggests that in Trogoderma granarium the release of the brain hormone may be under the direct control of temperature; the high the temperature the more release of products from the cells (see page 40).

As far as food is concerned, it is possible that in T. granarium food - besides its increasing the metabolic activity of the larva (Burges, 1960) and the synthesis of the secretory material - may also provide a further stimulus for release of the brain hormone. In Rhodnius, the meal of blood has three immediate effects; stretching the abdomen, nutrition of the tissues and liberation of moulting

hormone. This meal of blood initiates moulting in Rhodnius (Wigglesworth, 1963b).

Finally, it must be mentioned that in T. granarium the effects of temperature and food are not of course completely divorced from each other; thus synthesis of neurosecretory product requires the presence of food but the synthesis proceeds faster at the higher temperature. It is also possible that the release of hormone which it seems is governed primarily by temperature, may also be affected by nervous signals from gut distended by food or by the movement of the pharynx.

VI SECRETORY DYNAMICS OF A - CELLS AS STUDIED BY AUTORADIOGRAPHY

Introduction

Histological examination of the neurosecretory system in growing non-dormant larvae reveals that the A - cells contain small amounts of stainable secretion. In the case of dormant larvae the amounts of secretory product in the cells are very large. These observations suggest that when the neurosecretory cells contain small amounts of material they are at 'high rate' of release, producing hormones which bring about the moulting and metamorphosis. The presence of large amounts of neurosecretion in these cells suggests that the cells are at 'low rate' of release and the product is accumulated within the cells. The conclusion that the neurosecretory cells are at high rate of release when they contain small amounts of product and that they are at low rate of release when the product accumulates within them, is based largely upon circumstantial evidence. Therefore, it was desirable to attempt to ascertain this conclusion by a more direct proof. This was achieved by the use of radioactively labelled amino acid ^{35}S-cystine in an autoradiographic study.

Sloper (Sloper, 1958; Sloper *et al.* 1960) has shown that this method can give consistent measures of neurosecretory activity in the rat hypothalmo-hypophysial neurosecretory system. Highnam (1962c) also using ^{35}S-cystine, was able to compare the activity of the neurosecretory system of females reared with mature males against females reared without males in *Schistocerca gregaria*. Also Siew (1963) has used ^{35}S-cystine to compare the rates of secretory activities of the neurosecretory system in *G. tanaceti*.

The following experiments and the results must be regarded as preliminary. They are designed primarily to test directly the inferences as to secretory dynamics by the incorporation of radioactively labelled substrates into the A - cells of the brain and the sub-oesophageal ganglion, followed by autoradiographs.

Materials and technique

Six larvae were used to represent each non-dormant and dormant phase. The dormant larvae were chosen from those which have been kept at 25°C and supplied with food. ^{35}S-DL-cystine was obtained from the Radiochemical Centre, Amersham. The sample, having a specific activity of 0.119 mc/mg. was dissolved in 0.1 ml. of 0.8 NHCl and 2.6 mls. of Ringer's solution. One microlitre of the solution was injected by means of a micro-syringe into each larva. Thus each individual received about 0.5 uc. of ^{35}S. The larvae were then replaced in their previous environments.

Twenty four hours later, the larvae were fixed, sectioned and lightly stained in the usual way to bring out the A - cells. Then they were washed in distilled water, layered with the stripping film emulsion (Kodak AR 10) and the exposure carried out at 4°C. for 21 days in a light-tight box containing silica gel to absorb any sealed-in moisture. The autoradiographs were then developed with Kodak D - 19B developer according to the maker's specifications and fixed.

The numbers of silver grains per neurosecretory cells were counted under an oil immersion lens. The diameters of the nuclei were also measured. Silver grains were also counted in an adjacent area of the central nervous system equal to the area of the neurosecretory cells which have appeared in the specimens and used for counting. Thus relative activity of A - cells was calculated from the number of silver grains in A - cells over the number of silver grains in equal area of the "background". The latter was a mean of three readings of area of "background".

Results

All the larvae survived the injection and lived till fixation. Fixation and staining of the tissues before exposure to the film may well have leached much of the radioactive substance from the tissues. Nevertheless satisfactory results were obtained from three non-dormant larvae and from four dormant ones. The results are summarised as follows:-

Physiological condition of larvae	No. of larvae	Nuclear dia. (mean)	Mean no. of grains per cell	Mean no. of grains per equal background areas	Relative activity
non-dormant larvae	1	4.5 u	165	77	2.14
	2	5.0 u	219	110	1.90
	3	4.4 u	170	77	2.20
dormant larvae	1	2.3 u	96	86	1.11
	2	2.5 u	112	94	1.14
	3	2.5 u	89	74	1.20
	4	2.4 u	90	82	1.09

Each count given is the mean derived from cells which were stained.

Although this study of the uptake of labelled amino acids by the neurosecretory cells in T. granarium is still regarded as preliminary, appreciable results are obtained. These are summarised as follows:

1. In non-dormant larvae, incorporation of cystine has proceeded approximately twice as actively in the neurosecretory cells as in the non-secretory neurones and bodies which made up the 'background'. In dormant larvae, a slight difference is found of the order of 10%.
2. The relationship between nuclear volumes and activity is confirmed. The larger nuclei of non-dormant larvae are associated with greater activity than the smaller nuclei of dormant larvae.
3. The uptake of ^{35}S by the neurosecretory cells was correlated with the synthesis of neurosecretory material and their subsequent release. It is confirmed that in non-dormant larvae of T. granarium, when the neurosecretory cells contain small amounts of stainable material they are at a 'higher' rate release' than when they contain large amounts of stainable material. This is in agreement with the findings of Highnam (1962c) in the brain of S. gregaria and Siew (1963) in G. tanaceti. On the other hand, Delphin (1963) maintained that the secretory activity of the neurosecretory cells in the abdominal ganglia of S. gregaria, with smaller amount of product, is not greater than that with more secretion

VII EXPERIMENTAL INVESTIGATIONS INTO THE ROLE OF THE NEUROSECRETORY SYSTEM IN CONTROLLING METAMORPHOSIS AND DORMANCY

Introduction

The preceding studies have shown the spectacular changes of the neurosecretory system in response to the external environment. The following pages are concerned with experimental studies to ascertain the individual roles played by the components of the endocrine system in controlling metamorphosis and dormancy and the relationship between the various components.

Owing to the minute size of T. granarium larva, only four methods were employed, two of which gave satisfactory results. In the first, the brain or the suboesophageal ganglion was cauterised and the overall effect on metamorphosis and dormancy was studied. The second method was devoted to the use of farnesol, a substance known to simulate the effects of the juvenile hormone secreted by the corpus allatum (Wigglesworth, 1961, 1963). Thus evidence concerning the possible role of the corpus allatum may be deduced despite the impossibility of cauterising it. The third method is devoted to implantation experiments which unfortunately gave unsatisfactory results. Finally, in the fourth method, the larvae were ligatured behind the prothoracic segment for the purpose of studying the role of the prothoracic glands in inducing moulting.

I. Cauterisation experiments

Materials and technique

Forty non-dormant larvae of the sixth instar and forty dormant larvae were used in these experiments. Cauterisation was performed as described in pages 9 and 10. The larvae were then kept in petri dishes with perforated covers and food consisting of crushed wheat and bran was added. All were now raised at 30°C. and 60% R.H. Where possible, the remaining components of the neurosecretory system were examined at intervals after the cautery.

Results

a - Non-dormant larvae

i. Cauterisation of neurosecretory cells in the brain

75% of the larvae survived until inspection. The rest died either by handling or after the operation. The survivors moved and excreted slowly. They neither moulted nor pupated for two to four weeks but showed a slight decrease in length (Fig. 26).

Histologically, examination revealed that the remaining components of the neuro-endocrine system were inactive. The A - cell in the suboesophageal ganglion was conspicuously diminished, the cytoplasmic content decreased as well as the nuclear size. The C - cells did not appear in any of the specimens examined. No trace of brain secretion was found along the pathways; thus confirming that all the brain neurosecretory cells were cauterised. A few specimens showed the presence of a small amount of brain secretion stored in the corpus cardiacum. Both corpora cardiaca and allata were shrunken.

ii. Cauterisation of neurosecretory cells in the suboesophageal ganglion

The treated larvae were more active than those in which the brain had been cauterised. However, only 45% survived until fixation. None moulted or pupated for three weeks. The larvae, however, changed in shape (Fig. 27); the head became smaller, the body became shorter, the abdomen broadened and the abdominal segments greatly telescoped.

A histological examination revealed that the neurosecretory cells of the brain were apparently functioning at a low level of activity. The corpora cardiaca contained little brain neurosecretion and the corpora allata were shrunken.

An interesting feature occurred in those cauterised larvae. All the specimens examined showed that the connectives between the ventral ganglia were conspicuously folded, a fact presumably related to the greatly telescoped form of the abdomen.

b - Dormant larvae

Work described in the previous section demonstrated that when dormant T. granarium larvae were moved to a higher temperature, 30°C, and were provided with food, they soon broke dormancy and either moulted or pupated. The following experiments were designed to show whether the dormant larvae would still moult and pupate when moved to favourable environments, if the neurosecretory cells of either the brain or the suboesophageal ganglion were removed.

i. Cauterisation of neurosecretory cells in the brain

Twelve larvae out of twenty survived until inspection. None moulted or pupated for three weeks. Thus cauterisation of the brain neurosecretory cells impeded termination of dormancy in the larvae in spite of being in favourable environments.

ii. Cauterisation of neurosecretory cells in the suboesophageal ganglion.

Only 40% of the larvae survived the operation. None moulted or pupated for about two weeks when they were fixed. Histological examination showed that the brain cells were conspicuously depleted and the corpora cardiaca and allata were extremely shrunken.

2. Application of farnesol

Introduction

Farnesol has been shown in certain insects to have similar properties to Juvenile hormone (Schmialek, 1961: Wigglesworth 1961 and 1963; Dearden, 1964). In Rhodnius, farnesol caused retention of larval characters in moulting fifth-instar larvae, and brought about a partial reversal of metamorphosis in the moulting adult (Wigglesworth, 1961). In D. melanogaster, it caused delay in puparium formation and eclosion of adults (Dearden, 1964). It has also the same effect upon ovarial development in insects as the corpus allatum hormone (Wigglesworth, 1961). In Schistocerca gregaria, farnesol prevent resorption in some females and reduced it in others. (Highnam, et al., 1963). Farnesol has been applied by previous workers in three different ways; firstly, by smearing it

with the head of an entomological pin on either the contact cuticle or after abrasion of the cuticle; secondly by injecting it in the body of the insect; thirdly, orally, by incorporating it in food.

The following experiment is an attempt to investigate the effect of farnesol on metamorphosis and dormancy in T. granarium.

Materials and technique

Twenty sixth-instar non-dormant larvae and ten dormant ones were used in this experiment. They were smeared daily with farnesol (see general methods and technique) and maintained at 30°C with food.

Results

The larvae fed and moved normally for a few days and then their movement ceased and they tended to adhere to the wall of the petri dishes. The larvae became conspicuously darker and the setae became more brittle. They showed no signs of pupating during the two weeks in which they were observed. 15% of the treated larvae, however, showed some evidence of moulting; parts of the cuticle gave the appearance of new cuticel forming separated from the partially digested exocuticle by a lacuna.

The neurosecretory cells in the brain and the suboesophageal ganglion appeared to be in a state of low rate of release. The cells contained large amounts of secretion but their volume decreased (Fig. 28). Small amounts of brain secretion were detected along the pathways but the corpus cardiacum was full of brain neurosecretion (Fig. 29). The volume of the corpus allatum decreased, accompanied by a decrease in the cell size. At the end of the second week, the corpus allatum measured approximately 29,920 u^3 in volume compared with a normal size of 38,150 u^3.

These results were obtained when farnesol was smeared twice a day every day. A question then arises: would the larva moult or pupate if farnesol was smeared for slow periods? To answer this question, the following experiment was performed.

Twenty sixth-instar larvae were smeared twice a day

Every day, two larvae were isolated without any further treatment.

All the larvae which had been isolated during the first week pupated within 24 - 48 hours from the time of isolation. The pupae lived and developed to adults. In larvae isolated after more than one week's treatment, pupation and adult formation depended upon the period of treatment. The longer the period, the less likely was pupation and adult formation.

3. Implantation experiments

Mature non-dormant larvae and dormant ones were used. The mature larvae had their brain cauterised (see general methods and technique). The larvae were then kept at 30°C. and were provided with food till they recovered completely, usually 2 - 3 hours after the operation. These larvae represented the recipients.

Brains were dissected under Ringer's solution from donors, usually mature larvae. The recipient larvae were chilled for ten minutes and then placed dorso-ventrally on a depression made in a plasticine operating stage. An incision was made with a very sharp pin, made especially for this purpose, on the dorsal aspect of two abdominal segments. The brain was then inserted into the abdomen by means of a pair of jeweler's forceps. The wound was sealed with paraffin wax.

The same implantation experiment was performed on chilled dormant larvae without cauterising their brains. Active brains from non-dormant larvae were implanted in dormant larvae.

Results

Unfortunately these experiments did not give any satisfactory results. In the most successful experiments the operated larvae lived from 8-10 hours only.

4. Ligature experiments

To demonstrate the function of the prothoracic glands only ligature experiments could be employed owing to the small size of the insects and the impossibility of cauterising the glands without any

further damage to other tissues.

Materials and technique

Ten mature larvae were ligatured behind the first thoracic segment by means of sterilized cotton. They were then kept in petri dishes and were provided with food. The culture was raised at 30°C. and 70% R.H. The larvae were fixed at one week intervals, sectioned and stained with either Mallory's triple stain or Paraldehyde fuchsin, PF.

Results

Only one larva moulted and cast its skin after two weeks from the time of the operation. This larva did not decrease in size and its abdomen was extremely expanded. The other nine did not show any sign of moulting before fixation. Sections revealed the presence of food in a large quantity in the abdomen of the moulting larva whereas in the others, fixed at the same time, the abdomen contained very little.

Discussion

The foregoing results support the view that growth and metamorphosis in larval T. granarium are under the control of the hormones from the neurosecretory system and the juvenile hormone from the corpus allatum.

Cauterisation of the neurosecretory cells in the brain or cessation of their hormone production consequent upon the destruction of the suboesophageal ganglion, prevented moulting and metamorphosis in non-dormant larvae, thus confirming the earlier findings by many workers in a number of insects, e.g. Wigglesworth (1940) in Rhodnius, Williams (1946) in Hyalophora, Possompès (1953) in Calliphora and Nayar (1956) in Iphita.

Dormant larvae which had their brain neurosecretory cells removed did not break dormancy when they were moved to favourable environments. This suggests that the brain hormone exerts a controlling influence on the onset or termination of dormancy in Trogoderma. The role of the brain hormone in diapause has been demonstrated in pupal diapause of

Hyalophora cecropia (Williams, 1946), Platysamia (Van der Kloot, 1955, 1960, 1961); in the larval diapause of Chilo (Fukaya, 1955), Lucilia (Fraser, 1960) and in imaginal diapause of G. tenaceti (Siew, 1963).

The depletion of the neurosecretory cells in the suboesophageal ganglion after the removal of the neurosecretory cells in the brain suggests that the activity of the secretory cells of the ganglion is under the control of the brain.

The presence of the suboesophageal ganglion hormone in cauterised brain larvae neither caused moulting nor terminated dormancy. On the other hand, extirpation of the neurosecretory cell of the ganglion has been demonstrated to cause arrest of growth and metamorphosis, to sustain dormancy and to cause the neurosecretory cells in the brain to become inactive. It seems that the hormone produced by the suboesophageal ganglion does not govern the resumption of growth and yet its presence is necessary for the neurosecretory cells of the brain to release sufficient secretion to induce moulting and to function in response to the stimuli of the environment. This also raises the possibility that a feed-back mechanism from the suboesophageal ganglion may be involved to ensure a continual activation of the brain hormone in its response to the environment.

In T. granarium, cautery of either the neurosecretory cells of the brain or the neurosecretory cell in the suboesophageal ganglion produced atrophied or shrunken corpora cardiaca and allata. This suggests that the activity of the corpora cardiaca and allata is under a stimulus from both the brain and the suboesophageal ganglion. The corpora cardiaca/allata-suboesophageal nerve exists in T. granarium larvae and it is tempting to suggest that the ganglion stimulates the corpus allatum via nervous impulses. This is in agreement with the findings of Engelmann (1957) in Leucophaea.

Reproducing the effects of juvenile hormone, farnesol treatments provide evidence that metamorphosis in T. granarium is under the control of the activity of the corpus allatum, thus confirming the findings of other workers (see Wigglesworth, 1954, 1961, 1963 and 1964). In spite of the activity of the neurosecretory cells of the brain and the suboesophageal ganglion in growing larvae, the application of farnesol

prevented metamorphosis in those larvae and impeded termination of dormancy in dormant larvae even after they were removed to favourable environments. This suggests that excess of juvenile hormone in larvae of T. granarium has an overriding influence on the hormones secreted by the neurosecretory system. Implanting the corpus allatum of a 4th-stage larva of Rhodnius in a 5th-stage larva prevented metamorphosis in the latter and a 6th-stage larva was formed in spite of the activity of the brain (Wigglesworth, 1934, 1936). Other examples of such effects of the corpus allatum and the juvenile hormone secreted by it have been confirmed in many groups of insects (see Wigglesworth, 1954a, 1964).

An excess of farnesol has been demonstrated to cause an accumulation of neurosecretory material within the neurosecretory cells and the corpora cardiaca and to cause shrinkage in the volume of the corpus allatum. This suggests that an excess of juvenile hormone may act back on the neurosecretory cells and the corpus allatum. An excess of corpus allatum hormone can lead to an accumulation of neurosecretory material within the neurosecretory system and also an inhibition of the corpora allata activity in Periplaneta (Nayar, 1962). Also, farnesol was demonstrated to act back on the neurosecretory/corpus allatum system when it was smeared at early stages of oocyte growth, causing increased resorption in Schistocerca (Highnam, 1963).

When farnesol was applied periodically, different results were obtained according to the length of the period of farnesol treatment. Thus these results indicate that the presence of juvenile hormone - or in these experiments a substance mimicking its effect - either delays or completely inhibits metamorphosis depending on the amount of this hormone circulating in the blood; and in its absence the larvae moult and develop pupal characters. This effect can explain why some larvae moult to extra larval stages while others moult to form pupae.

Ligature experiments suggest that the thoracic glands in T. granarium are probably activated by the brain hormone. The larva which had moulted apparently consumed more food, judging by the appearance of the abdomen and the quantity of food present in the gut. Food has been shown in the present work to play an important role in the liberation of brain hormone. Thus in that larva which moulted, the neurosecretory cells secreted their hormones and consequently the prothoracic glands

were activated and subsequently moult occurred. The fact that food was present in large quantity in the abdomen and the consequent expansion which occurred to it indicate that the ligature was not tight enough. Thus moult occurred in all parts of the body. Retaining the larval characteristics after moulting may be due to the presence of the juvenile hormone.

The lack of food in the abdomens of the others supports that in these cases the ligature was probably sound. Such a ligature prevented the larvae from feeding and consequently no brain hormone was liberated and hence no moulting occurred. The prothoracic glands have been shown to be only functional after being activated by brain hormone (Williams, 1946, 1948, 1952 and 1955).

Finally it must be mentioned that the ligature experiments performed in the present work are rather preliminary and further experiments are needed.

VIII THE NEUROSECRETORY SYSTEM IN THE PUPA

Introduction

The neurosecretory system of the pupa, as in the larva, consists of neurosecretory cells in the brain and suboesophageal ganglion together with the corpora cardiaca and allata. However, the number, size and type are slightly different. No neurosecretory cells were found in any of the other ganglia. This section is divided into

1. Histological studies
2. Experimental studies

Rearing technique

Trogoderma granarium pupates enclosed in the last larval skin. At the last ecdysis the larval skin splits dorsally from the posterior margin of the head to the fifth or sixth abdominal tergite, but is not cast off, the pupa remaining within this skin for the whole of its life.

Four day old mature larvae were kept in plastic petri dishes with perforated covers. They were provided with food which consisted of whole wheat grains and bran, and the culture was raised at 30° C. and 70% R.H. The larvae were examined at 3 hour intervals. Those which had their skins split were considered to be pupating.

Pupae were fixed at 24 hour intervals from this stage.

Results

1. Histological studies

a - The neurosecretory cells in the brain

Two conspicuous groups of neurosecretory cells have been found in the pars intercerebralis region. Each group of cells is lodged in each hemisphere of the brain, but in some sections there is indistinct

demarcation of the two groups and they appeared to be a single cluster. After staining with PF, two types of cells, A and B, were identified. In each groups, there are ten A - cells staining deep purple and 4 - 5 B - cells, staining bright bluish green, located among the A - cells.

Two other groups of neurosecretory cells, lateral groups, one in each hemisphere are located dorso-laterally in the protocerebral lobe just outside the parsintercerebralis of the brain. Each lateral group of neurosecretory cells is composed of 2 - 3 A - cells. They have the same tinctorial affinity and the histological picture of the cytoplasmic inclusions of A - cells of the median group using PF, CHP and azan.

The neurosecretory cells of the brain showed a definite pattern of activity throughout the pupal life (see Table 4.).

In 0 - 24 hour old pupae, the neurosecretory cells were smaller compared with those of the older pupae. The rate of synthesis of the secretory product seemed to be at the same level as the rate of release. The cells and the pathways contained secretory products at the same level. Small amounts of the brain secretion were found in the corpus cardiacum (Figs. 30 ,31 , & 32 .)

In 24 - 72 hours old pupae, the neurosecretory cells were at their peak. They were larger than those of the younger and older pupae. The rate of synthesis, however, appeared to be higher than the rate of release of secretory products. The cells contained a large amount of neurosecretion whilst the amount of secretory product carried along the axons was small. The corpus cardiacum contained a large amount of brain secretion. (Figs. 33 ,34 , & 35 .)

In 72 - 96 hours old pupae the brain cells became slightly smaller than the previous stage and they contained a smaller amount of secretory products. However, the amount of brain secret carried along the axons was larger than in the two previous stages. The corpus cardiacum contained very little brain secretion. (Figs. 36, 37 and 38.).

b - <u>The neurosecretory cells in the sub-oesophageal ganglion (Fig. 39)</u>.

Only one A - cell was found in the suboesophageal ganglion. The five C - cells found in the mature larvae disappeared in the pupa. The A - cell lies in the mid-ventral line of the ganglion.

The rate of synthesis and release of the A - cell followed the same pattern as the brain cells (see Table 4.).

Table 4. The qualitative and quantitative changes in the neurosecretory system of the pupa throughout its life, based on examination of 5 specimens at each stage. The same arbitrary categories and the arithmetic symbols used in describing the activity of the larval system are used.

Neurosecretory system		Age in hours		
		0 - 24	24 - 72	72 - 96
Brain cell measurement in u	A cells	13 x 7	16 x 10	14 x 10
	nucleus	4·5 x 4·5	4·5 x 4·5	4·5 x 4·5
	B cells	7 x 8	10 x 9	10 x 8
	nucleus	3 x 3	3·5 x 3·5	3·5 x 3·5
Stages	A cells	III	IV & V	II
	B cells	III	IV	II
Brain secretion along axons		+ +	+	+ + +
Brain secretion in corpus cardiacum		+ +	+ + +	+
Suboesophageal ganglion cell measurement	A cell	14 x 8	15 x 8	15 x 8
	nucleus			
Stage		III	V	II
Cell secretion along axons		+ +	+	+ + +
Volume of corpus cardiacum in u^3		176900	159700	198900
Volume of corpus allatum in u^3		182460	182000	215000

e - The corpora cardiaca

As in the larva, the corpus cardiacum is connected to the brain by the nervi corpori cardiaci NCCI and NCCII, and it consists of the two types of cells, the 'chromophile cells' and the chromophobe cells. However, in the pupa it was possible to define a cortex and a medulla owing to the cells' distribution. The 'chromophile cells' are situated in the peripheral regions, thus defining the cortex. The second type, the 'chromophobe cells' are scattered in the matrix of the gland in the medullary zone.

The size and the inclusions of the gland fluctuated throughout the pupal life. However, it attained the largest size in the pre-eclosion period (see Table 4. and Figs. 32, 35 and 38.).

d - The corpora allata

The corpus allatum is a compact body invested by a thin connective membrane which became thicker than that of the larva. It stained intensely bluish-green with PF. Some axons appear to leave the corpora cardiaca and ramify among the cells of the corpora allata, being dilated locally with globules of neurosecretory material. As in the larva, the corpus allatum consists of one type of cell but with well defined boundaries all throughout the pupal life.

There was no spectacular change in the volume and the inclusions of the gland compared with those in the neurosecretory cells and the corpus cardiacum. The gland appeared to maintain, more or less, the same activity throughout the whole pupal life (see Table 4. and Figs. 32, 35 and 38).

2. Experimental studies

As with the larva, only two kinds of experiment could be employed owing to the very small head of the pupa: cauterisation of the neurosecretory cells and the application of farnesol as an extract mimicking the effects of Juvenile hormone as secreted by the corpus allatum.

A. Cauterisation experiments

Materials and technique

Two or three day old pupae were used. The pupa was taken out delicately from the larval skin through the split, the skin remaining attached posteriorly to the body. The pupa was put on the operating stage with its ventral side uppermost as the head is situated underneath the prothorax. The pupa was then held in place by a strip of filter paper pinned to the stage by two fine entomological pins and cauterisation was carried out. The thin pad of plasticine used in fastening the larva to the operating stage was found fatal to the delicate body of the pupa (see general methods and technique). In the case of the pupa, also, a temperature of 200° - 300°C. was found rather satisfactory for cauterisation. An equal number of insects taken as controls were dissected but not cauterised.

i. Cauterisation of the neurosecretory cells in the brain

Twenty-four per cent of the operated pupae survived for 5 - 6 days. None became adult and nothing changed in the appearance of the pupa except that they became slightly smaller.

Examination of the head region revealed greatly shrunken corpora cardiaca and allata. Corpora cardiaca, in particular, were often difficult to find owing to their atrophy.

The control pupae all developed into adults in due course.

ii. Cauterisation of the neurosecretory cells in the sub-oesophageal ganglion

Owing to the difficulties of the method, only three pupae out of 30 were successfully cauterised. The rest were killed due to handling. Two pupae lived for one day and the third survived for five days. The pupa did not become adult.

Examination of the head region revealed depleted brain cells and shrunken corpora cardiaca/allata.

Eighty per cent of control pupae died after one day. The survivors became adult within 4 - 5 days after the operation.

B. Application of farnesol

Materials and technique

Pupae of different ages were smeared daily with farnesol after widening the dorsal split of the cast larval skin by means of fine entomological pins (see general methods and technique).

Results (Figs. 40 & 41)

The result is summaries in the table below:

Table 5.

Age and no. of treated pupae		Adult formation
0 - 24 hours old	1	nil
	2	nil
	3	nil
	4	nil
24 - 48 hours old	1	nil (little differentiation)
	2	two days delay
	3	nil (little differentiation)
	4	two days delay
48 - 72 hours old	1	nil (little differentiation)
	2	three days delay
	3	nil (intermediate)
	4	nil (little differentiation)
72 - 96 hours old	1	one day delay
	2	deformed adult
	3	adult without delay
	4	one day delay

About 63% of the treated pupae did not develop to adult. 40% of these pupae, however, showed little further differentiation of

adult characters (Fig. 40a), i.e. increase in size of the head and elytra, darkening of the cuticle and appearance of a few adult hairs. These changes are more likely to occur in treated pupae of 24 - 72 hours old. About 6% of the treated pupae developed to intermediate stages (metathetely, Fig. 40b), i.e. the head, prothorax and legs were completely of the adult, but the abdomen, elytra, hindwings were in pupal stage. One treated pupa developed into a deformed adult (Fig. 41). The intermediate stages and deformation occur in treated pupae of 72 - 96 hours old. The rest of the treated pupae, about 37%, developed to normal adults.

Discussion

Histological examination of the neuro-endocrine system of the pupa throughout its life revealed a correlation between the activity of the neurosecretory system and metamorphoses. During the first days of the pupal life, the neurosecretory system was active synthetically, but at low rate of release. Thus a large amount of brain secretion accumulated in the corpora cardiaca. Coinciding with the pre-eclosion period, the neurosecretory cells were at high rate of release and the amount of brain secretory products in the corpus cardiacum was small. These observations suggest that the amount of the brain secretory products circulating in the blood is higher during the pre-eclosion period than in the earlier stage and pupal - adult moult depends on the activity of the neurosecretory system.

The experimental work also indicates that the pupal - adult moult depends on the activity of the brain. Cauterisation of the neurosecretory cells in the brain of pupa *T. granarium* prevented the pupa from developing to adult. The brain has been shown to have the same action in pupae *Platysamia* and *Telea* (Williams, 1946) and *Mimas tilia* (Highnam, 1958).

Cauterisation of the neurosecretory cell of the sub-oesophageal ganglion prevented pupal-adult moult. On the other hand, its presence in cauterised brain pupa did not cause development in the pupa. This suggests that the pupa - adult moult is under the control of a combined action from both the brain and the suboesophageal ganglion.

The atrophy and shrinkage of corpora cardiaca and allata in the absence of neurosecretory cells of the brain and the suboesophageal ganglion indicates that these glands are under the control of the neurosecretory system.

Farnesol experiments revealed results similar to those obtained after treating the larva with farnesol. Farnesol reproduced the effects of the juvenile hormone and prevented some pupae from developing to adults. An interesting point, however, has arisen after treating pupae with farnesol, namely, the control of timing and concentration of farnesol and the reaction of the pupa towards these two factors.

Larval, pupal and adult moults are associated with progressively smaller amounts of juvenile hormone (Wigglesworth, 1954). It is likely that a similar relationship holds for T. granarium. Thus, when farnesol was applied to young pupae, there was already much juvenile hormone in the body and excess of it would not disturb the balance of hormones. Consequently, the pupa did not develop into an abnormal adult. Two days later, the juvenile hormone in the body of the pupa decreased; so excess farnesol apparently disturbed the balance of hormone; thus intermediates were obtained. At later days where much less juvenile hormone was in the body of the pupa, farnesol could not prevent metamorphoses and the pupae developed into adults. A slight disturbance in the balance of hormone, however, occurred in one of the beetles. Thus an adult with a deformed wing occurred.

Abnormalities and intermediates have been demonstrated, by several workers, to result from upsetting the balance of hormones in insects (see Wigglesworth, 1954).

Finally, it must be mentioned that the normal growth and metamorphosis of T. granarium depend at each stage on a competition and a delicate balance between the neurosecretory system hormone and the corpus allatum hormone. It is for this reason that farnesol compounds, mimicking juvenile hormone, may prove of interest as possible chemicals for insect control. Evidence has been presented by Williams (1964) showing that juvenile hormone is the most promising candidate for us as an insecticide.

IX REPRODUCTION AND NEURO-ENDOCRINE RELATIONS IN ADULT T. GRANARIUM

Introduction

In the previous sections the interrelationships of the brain and other endocrine organs and their effect on growth and metamorphosis and diapause in the larvae and pupae have been discussed. This section is devoted to an account of the relationships and the effects of the brain and endocrine organs on reproduction in both adult female and male.

A vast amount of data has been published on the neuro-endocrine-reproduction relations in female insects showing that ovarial maturation and oviposition are controlled by different levels of hormonal activity of the neurosecretory system; but virtually nothing has been published about the male.

It was in 1936 that Wigglesworth first observed that the corpora allata were necessary for the production of ripe eggs in the adult female and for producing secretion in the accessory glands in o Rhodnius. Since then, many authors have come to the conclusion that the corpora allata are necessary for yolk deposition, e.g., in Melanoplus differentialis (Pfeiffer, 1939; 1945), Calliphora erythrocephala (Thomsen, 1940; 1942), Dytiscus marginalis (Joly, 1945), Leucophaea maderae (Scharrer, 1946a, b), mosquitoes (Clements, 1956), Oncopeltus fasciatus (Johansson, 1958), Dermestes maculatus (Ladduwahetty, 1962), Schistocerca gregaria (Highnam, 1962 and Highnam et al. 1963), and recently in Galeruca tanaceti (Siew, 1963).

On the other hand, egg maturation proceeds without interruption in allatectomised females of Carausius morosus, unless the glands are extirpated at an early nymphal stage (Pflugfelder, 1937a, b; 1939). Similarly, in certain Lepidoptera, e.g., Bombyx mori, the corpora allata are apparently not required for egg production (Bounhiol, 1938, 1942; Fukuda, 1944). Also, Thomsen (1952) observed that a certain number of Calliphora females deprived of their corpora allata developed yolk in their oocytes. Possompes (1953) showed that if the gland was extirpated in the last larval stage, the resulting adults generally

laid viable eggs. However, in some of these cases the developing pupae have fully mature eggs, and probably there is still a high level of the hormone produced by the corpora allata in the body (see Williams, 1956a).

The role of the corpus cardiacum in controlling reproduction is not yet fully understood. The gland extracts have been shown to stimulate contractions of gut muscles and malphigian tubules (Cameron, 1955), to stimulate other centres, for instance pericardial cells, to produce a pharmacologically active agent (Davey, 1961, 1962) and to stimulate, then to depress, nervous activity (Ozbas and Hodgson, 1958; Milburn et al., 1960).

It has been shown that copulatory reflexes in mantids and cockroaches are controlled by efferent-motor activity in the phallic nerves. Roeder (1935) observed that the decapitation of these male insects by the females preceding copulation elicits very intensive copulatory reflexes from the headless males. The inhibition of these reflexes is normally controlled by the suboesophageal ganglion. If a corpus cardiacum extract is applied to an intact nerve in a cockroach, bursts of nerve impulses develop in the phallic motor nerves mimicking the effects produced by decapitation. The corpus cardiacum is thus thought to restrict the activity of the 'inhibitory' centre in the suboesophageal ganglion (Milburn et al., 1960).

Highnam (1962a) demonstrated experimentally that removal of the corpora cardiaca will prevent the development of the terminal oocytes in the ovaries of Schistocerca gregaria. However, in the absence of brain secretion, the corpora cardiaca do not inhibit ovarial development. The corpus cardiacum in G. tanaceti decreases in volume after each oviposition, suggesting that it has an active role in oviposition (Siew, 1963). Siew speculated that the corpus cardiacum might release its secretion to restrict an 'inhibitory' centre, thus eliciting well-conditioned ovipository reflexes which are necessary for the successful laying of batches of eggs at fairly lengthy time intervals.

It was not until 1952 that Thomsen showed experimentally that the medial neurosecretory cells in the brain of Calliphora exert an overall controlling influence on ovarial development. The influence of the neurosecretory cells appears to act primarily to activate the corpus allatum and, secondly, to promote ovarial development independently of

the corpus allatum by its secretion. Gillett (1958) demonstrated that the ovarial development in Aedes aegypti depends on the release of a hormone from the brain. Similarly, transplantation of medial neurosecretory cells from a 'gravid' female of Iphita limbata Stal to an immature one proved that these cells play a major role in inducing oviposition (Nayar, 1958a).

In Schistocerca gregaria ovarian development is controlled by the neurosecretory cells of the pars intercerebralis (Highnam, 1961; 1962a, b and c). His conclusion was based on a histological study of the neurosecretory system, and was confirmed experimentally. Ladduwahetty (1962) also proved experimentally that oocyte maturation is restricted in young females of Dermestes maculatus deprived of their medial neurosecretory cells. Similar results have been obtained in Galeruca tanaceti by Siew (1963).

Conversely, the medial neurosecretory cells do not exert a control over ovarial development in phasmids (Dupont-Raabe, 1952; 1954). However, Dupont-Raabe (1951, 1954, 1956) and Arvy and Gabe (1952a, b; 1953a, b,c,d) working on a variety of insects have correlated histological differences in the appearance of neurosecretory cells with the state of ovarial development. In Oncopeltus fasciatus, egg development proceeds in the absence of medial neurosecretory cells although the fecundity and length of oviposition periods are reduced (Johannson, 1958).

As far as the lateral groups of neurosecretory cells are concerned, some investigators showed that they collaborate with those of the medial groups to produce a single or multiple hormone, capable of inducing ovarial development (Williams, 1948b; Siew 1963). However, Thomsen (1952) maintained that the medial neurosecretory cells are the main neuro-endocrine centre as cauterisation of the lateral cells only partially retards egg development in Calliphora erythrocephala. Ladduwahetti (1962) also showed that the lateral groups of neurosecretory cells in Dermestes maculatus are unable to influence the maturation of ovaries independently, and she ascertained that A - cells (median group) are the main source of hormones for oocyte development.

An interesting relationship between the neuro-endocrine organs and the reproductive cycle has also been traced in Leucophaea maderae and Diploptera punctata. Engelman (1957; 1958; 1959; 1960)

maintained that during pregnancy, the ovaries are quiescent as a result of inactivity of thecorpora allata, which in turn are restrained by the brain via nervous pathways. His experiments also demonstrated that the corpora allata are reactivated when the egg-case is laid, via a stimulatory centre in the suboesophageal ganglion. The corpora allata could be activated during pregnancy by the removal of ootheca from the brood sac. This activation, however, did not take place if the eggs were implanted into the body cavity immediately after the removal of the ootheca. This indicates that the developing eggs in the brood sac release a substance, which causes the brain to inhibit the corpora allata. Engelman's experiments further indicate that the genital apparatus transmits nervous impulses to the brain for the control of the corpora allata.

Ovariectomy of immature females of Schistocerca gregaria causes a precocious accumulation of material in the pars intercerebralis - corpora cardiaca neurosecretory system (Highnam, 1962). However, Delphin (1963) contradicted Highnam's results and showed experimentally that, in the early adult of Schistocerca gregaria, ovariectomy has no effect on the neurosecretory cells of the brain and the suboesophageal ganglion.

With respect to the relationship between the neurosecretory cells of the ventral ganglia and egg maturation, except for Delphin's (1963) and Siew's (1963) work, nothing is known about their function. Delphin maintained that the A - cells in the ventral ganglia of female Schistocerca gregaria undergo changes in secretory activity (as judged by histological criteria), which are correlated with maturation and oviposition. He added that the last abdominal ganglion appears to play a special role in ovarian maturation, through hormonal activity. However, the process seems to involve interaction with the neurosecretory cells of the pars intercerebralis, and he was not certain whether neurosecretion takes place in the last abdominal ganglion. Delphin concluded that the control of oviposition from abdominal ganglia is a nervous one. Siew (1963) found that the suboesophageal ganglion in Galeruca tanaceti does not initiate oocyte differentiation, and yet its presence is necessary for the neurosecretory cells of the brain to function in response to the stimuli of the environment. He showed experimentally that ovipositing females will not oviposit, even when they contain mature oocytes, if deprived of the neurosecretory cells of the ganglion.

The foregoing resume indicates that a brain - suboesophageal ganglion - corpora cardiaca/allata - reproduction mechanism operates in the above insects. The present work was undertaken to determine whether this conclusion could also be applied to T. granarium.

1. The anatomy, histology and growth of the reproductive systems at different stages of the adult life

When the adult beetle emerges, the pupal skin is cast off and remains within the posterior end of the larval skin. In the present work it was found that at 33°C. and 65% R.H., the adult remains enclosed within the larval skin for about 24 - 30 hours for males and from 30 - 36 hours for females. Within this period the cuticle hardens, pigmentation takes place and apparently maturation is also completed, as copulation takes place immediately after the adult leaves the cast larval skin. A short pre-oviposition period of about 24 hours followed copulation and the oviposition period is completed in about four days at 33°C. and 65% R.H. The majority of the eggs are deposited in the first two days of the period of oviposition and then production of eggs falls sharply on the next day and gradually for another two days. The female lives one or two days longer without laying eggs before death. Unmated females live longer than males and the latter outlive fertilised females.

Rearing technique

The sex of the pupae may be determined by their size. The male pupa is much smaller than the female. So new pupae of both sexes were transferred separately in petri dishes with perforated covers. They were raised at a temperature of 33°C. and 67% R.H. As the length of the pupal life is about four days for males and five days for females in this environment, examining the pupae on about the first three days was omitted, thus decreasing the disturbance and exposure of the pupa to a low temperature. Then pupae were examined every three hours until the pupal skin was cast off and the white adult appeared. These adults were isolated and tagged. The sex was confirmed by examining either the genitalia or the antennae.

Some of the females and males were fixed at the time of emergence, adults from 0 - 3 hours old, then at three hour intervals until the adults left the cast larval skins, then at 24 - hour intervals until death. The rest were paired, put into 1" x 2" tubes and covered with muslin. A strip of black paper folded concertina-fashion was placed in each tube to serve as an oviposition site. Although adult T. granarium do not feed, a thin layer ofwhole wheat grain was added as some adults prefer to lay their eggs on grains. Males and females were fixed at three hour intervals till copulation took place. Couples were fixed during copulation and then at every 24 - hour intervals throughout the pre-oviposition, oviposition and post-oviposition periods.

After fixation, the heads were parted from the abdomens and were kept separately for a study of the neurosecretory systems.

Results

a - The female reproductive system

i. Morphology (Fig. 42)

The female reproductive system consists of two ovaries lying on either side of the digestive tract and intimately close to it. Each ovary is surrounded by a fat body and composed of six telotrophic ovarioles. Each ovariole is an elongate tube tapering apically to form a thread-like terminal filament. The six terminal filaments of one ovary combine to form a common delicate strand of tissue and this unites in turn with its counterpart from the ovary on the opposite side, to form a single median ligament. The latter is attached either to the fat body or to the body wall, thus maintaining the ovaries in position within the body cavity. Immediately posterior to the terminal filament lies the second region of the ovariole, the germarium. This forms the apex of the ovariole and from it the nutritive cells and the oocytes originate. Below the germarium lies the vitellarium which occupies the largest region of the ovariole. It consists of a series of developing oocytes, the largest and oldest of which is the nearest to the lower end. Below the vitellarium lies a stalk-like tube, the pedicle, from which the mature egg is plugged

to the lateral oviduct. The six independent pedicles of each ovary become attached together posteriorly and communicate with the corresponding internal part of the lateral oviduct, in which they pour the developed oocytes. Posteriorly, the two lateral oviducts join the common median oviduct, which eventually opens into the vagina. The latter does not differ from the median oviduct and it is merely an extension of the median duct. Posteriorly, the vagina extends into the ninth segment to open there by way of the definitive genital aperture. Anteriorly, the vagina develops a pouch-like organ, the bursa copulatrix, at the junction of the median common oviduct with the vagina. The internal wall of the bursa copulatrix is sclerotised by a dorsal and ventral elongated sclerite and a small unpaired plate. The bursa copulatrix leads to a narrower sac, the spermatheca, which is surrounded by a mass of fatty tissue. The blind end of the spermatheca is heavily sclerotised forming a capsule. A long spermathecal gland, which lies dorso-laterially to the left ovary and is lodged in the fat body, opens closely to the capsule.

ii. Histology

The terminal filament

This is an extremely delicate conical structure (Fig. 43 a and b) with a slightly expanded basal region which narrows gradually into a slender protoplasmic strand. In the basal region there is a transverse septum which separates the terminal filament from the apex of the granarium. The outer wall of the filament is an epithelial membrane which, in fact, is the outer epithelial sheath that covers the entire ovariole from the tip of the filament down to the pedicel. It is a fragile elliptical sheath with small ovoid nuclei. Beneath the outer epithelial sheath there is a thin membrane enclosing the syncytial cytoplasm of the filament, which contains large, irregularly arranged nuclei. At the base of the filament, these nuclei are tightly packed whereas at the anterior end they are scattered. A fine membrane separates this inner epithelium from the protoplasmic core. As the adult becomes older, this membrane disappears.

The germarium

This has an oval shape and is covered with two epithelial sheaths, an outer sheath which is the continuation of the outer epithelial sheath mentioned above, and an inner epithelial sheath, which corresponds to the inner envelope of Bonhag (1955). The inner epithelial sheath extends posteriorly covering all the vitellarium up to the pedicel. However, the cells are not visible as a continuous layer throughout the ovariole. They are elliptical and their nuclei appear scattered along the outer wall of the germarium, directly beneath the outer epithelial sheath. They can also be seen lying at the constrictions between the follicles and at the base of the last follicle. Inside these two sheaths lie different types of cells; trophocytes, which occupy the greater part of the germarium, oogonia, young oocytes, prefollicular tissue and interstitial cells.

Three types of trophocytes have been observed; mononucleate, binucleate, and multinucleate. The former is abundant and appears more compactly arranged in the distal region than those of the central and proximal parts. In the apex of the germarium (Fig. 43) the mononucleate trophocytes are subspherical cells with relatively large, spherical nuclei. They are smaller than the other two types. In the middle and the posterior regions of the germarium (Fig. 44) the trophocytes gradually become larger with larger nuclei and a few binucleate and multinucleate trophocytes amongst the mononucleate forms. The increase in size of trophocytes is accompanied by decrease in their number. The number and size of the nuclei are not fixed. It is believed that the increase in size and the decrease in number of the nuclei are partly the result of a very unusual process namely fusion of anterior trophocyte nuclei (Bonhag, 1953). Although there is this variation in size, it is impossible to establish specific zones on this basis. The most characteristic feature of the trophocyte nuclei is that they contain coarse chromatin aggregates which are more deeply stained in the distal trophocytes than in the central and proximal ones. These nuclei are surrounded by a thin layer of cytoplasm which in the posterior half of the germarium often exhibits an irregular outline. Some of these trophocytes have cytoplasmic projections and from these cells the nutritive cords originate (Fig. 44). Many

of these trophocytes were found in the young females, but as the adult grows older, the number of such trophocytes decreases. The nutritive cords are attached to the developing oocytes before they leave the germarium and enter the vitellarium. They pass from one oocyte to another and from one follicle to another through spaces in the follicular epithelium and they also penetrate the interfollicular cell masses in the same way (Figs. 45 and 46). These nutritive cords represent the bridges through which the nutritive contributions of the trophocytes pass to the developing oocytes. These nutritive cords were easily seen in immature adults and in adults in a pre-oviposition period, but as adults grow older these cords become difficult to see and finally disappear entirely by the end of the period of oviposition.

Besides the trophocytes, there are small oval cells embedded in the cytoplasm of the germarium. These cells are the interstitial cells (Fig. 44). The cell boundaries are seen but only with great difficulty in the immature adult and in the anterior region of the germarium of a mature adult. However, as they orientate themselves posteriorly, cellular boundaries become quite clear. In the posterior region of the germarium these cells aggregate and form the prefollicular tissue (Fig. 44) consisting of many layers of interstitial cells in the immature adult; the layers decrease as the adult becomes older. Some of this prefollicular tissue orientates itself around the developing oocytes to be transformed into the definitive one-layered follicular epithelium.

Among the prefollicular tissue and trophocytes, embedded in the cytoplasm, oogonia and young oocytes are found. They are in great numbers in the young adult and they were found throughout the germarium. As the adult grows older, the number of the oocytes and oogonia decreases and they are frequently seen in the posterior part of the germarium. The oocytes, with orientated prefollicular tissues around them, gradually form the characteristic follicles of the vitellarium.

The vitellarium (Fig. 42)

The tubular region of the ovariole lying posteriorly to

the germarium, this zone contains a series of growing oocytes or follicles arranged in a single row, the youngest being nearest to the germarium. In the youngest follicles, the small oocyte is surrounded by several layers of small celled follicular tissue (Fig. 45). Posteriorly, the cells and the nuclei of this tissue show gradually better defined orientation and form a single layer of columnar cells fitting closely together around the growing oocyte (Fig. 47). In older follicles the columnar cells show a marked increase in size characterised by a further increase in the size of the nuclei; the cells become cuboidal with spherical nuclei (Fig. 48). Each nucleus contains small particles and large aggregates of chromatin and a dark-stained nucleolus. Finally around the oldest follicle, the cells flatten out and broaden, forming a thin epithelial layer which contains flattened oval nuclei (Fig. 49). As the epithelium is stretched by the increasing size of the eggs, the epithelial cells of the older follicles become separated from each other and considerable openings appear in stained ovarioles. No mitotic figures were discernible in the columnar epithelium. The transition of the epithelium from a columnar, through a cuboidal, to a flattened type seems to be an accommodation to changes in volume of the growing oocyte. It appears also that this transformation may arise as a result of the loss of mitotic activity of the follicular nuclei and hence any accommodation to the increase in volume of the developing oocytes could only be made by alteration in the size and shape of the follicular epithelium.

As the prefollicular cells accompany the growing oocytes to form the follicle tissue, some of them become wedges between successive follicles forming the inter-follicular tissue (Fig. 47 and 48). They do not differentiate, but always retain the prefollicular characteristics. The inter-follicular tissue is thicker in young adults than in older females. The mass of prefollicular cells which lies at the posterior end of the vitellarium separating it from the pedicel, form the so-called epithelial plug (Fig. 49). At the end of ovulation and after the previous egg has been discharged, the epithelial plug breaks down, letting the discharged egg pass into the pedicel. When this is accomplished the follicle anterior to the ruptured follicle becomes the terminal one and the last mass of interfollicular tissue assumes the function and position of the epithelial plug. The collapsed wall of

the discharged egg follicle contracts and the cells show signs of degeneration to produce a formless mass of cells. This mass is the 'Corpus luteum', not to be confused with the resorption body in virgin females (Figs. 49 and 50; see also page 75).

The pedicel

This is the last region of the ovariole connecting the vitellarium with the lateral oviduct. Histologically, it is a tubular structure with simple columnar epithelial walls covered externally by the continuation of the outer epithelial sheath.

iii. Quantitative and qualitative changes of female reproductive organs at different stages of adult life history

The reproductive system in female T. granarium changes so rapidly that in practice it was found that error of half an hour in sectioning or dissection might give a different result (see Table 5.),

Table 5. Measurements of female reproductive organs at different stages based on examination of 5 - 7 females of each stage

Reproductive Organs	0-3 hrs old	Fully mature unmated and mated female	Pre-oviposition period	Oviposition period	Post-oviposition period
Diameters of germarium in u.	233 x 94	328 x 112	350 x 130	350 x 135	110 x 27
Diameters of largest trophocyte in u.	9 x 9	11 x 11	13 x 13	13 x 13	7 x 7
Length of vitellarium in u.	211	1100	1600	1800	157
Diameters of terminal oocyte in u	90 x 50	180 x 290	185 x 300	195 x 300	72 x 157
Width of lateral oviduct in u.	70	100	120	125	40
Length and width of spermathecal gland in u.	450 x 100	650 x 135	690 x 180	680 x 170	350 x 90
Condition of spermatheca	empty	empty of spermatozoa	full	full	empty

In newly-emerged females (nil - 3 hours old) the reproductive system extends forwards only as far as the third abdominal tergum. Each ovary consists of six small ovarioles. The vitellarium consists of one to two developing oocytes (Fig. 51).

As ovarial development proceeds, a slight and gradual increase occurs in the germarium, pedical, lateral oviduct and the spermathecal gland. The vitellarium, however, increases more rapidly both in length and number of developing oocytes. Different stages of growth could be found not only between the two ovaries but also between the ovarioles of one ovary. The basal oocytes of 3 to 4 ovarioles of each ovary develop faster than the corresponding oocytes of the other ovarioles and contain different amounts of yolk deposits. By the time the female leaves the larval skin, each ovariole consists of eight developing oocytes and the two ovaries occupy a large portion of the abdomen and run forwards covering nearly the whole length of the metathorax (See Fig. 42).

Twenty-four hours later, in unmated females, 5 - 7 basal oocytes are collapsed (Fig. 52) having lost their opacity as the yolk was resorbed and deeply pigmented structures are formed. Moreover, 3 or 4 of the penultimate oocytes show different signs of yolk resorption. The deeply pigmented structures found at the bases of the ovarioles are comparable with the resorption bodies described in Locusta migratoria by Singh (1954, 1958), Schistocerca (Singh, 1954; Lusis, 1963) and in Dermestes (Ladduwahetti 1962). Yolk resorption continued so rapidly that 50% of the number of eggs were absorbed within the next 48 hours.

In females reared with males, maturation was completed in a shorter time than in those kept isolated. The females stayed in the larval skin for 27 - 30 hours; whereas in isolated females they stay about 48 hours in the larval skin till maturation is completed. After copulation, the ovaries become large and irregularly situated in the fully-expanded abdomen. Aceto-carmine squashed preparations of the spermatheca gave a reliable method of determining the presence of spermatozoa, the sperm heads being stained red with the aceto-carmine.

During the pre-oviposition period, which lasts for about one day, fully mature basal oocytes were always found and the spermatheca contained large amounts of spermatozoa.

Throughout the oviposition period, which lasts for about four days, egg development is continuous; oviposition follows ovulation and spermatozoa are always found in the spermatheca. On the first two days of the oviposition period, ovulation is at the highest rate and from 20 to 32 eggs were laid by a female. In females examined on these days a single egg may generally be found in each lateral oviduct. The eggs are not apparently stored in the calices for any length of time as, in all the females examined, there was never more than one egg in either of the lateral oviducts. As the egg passes out of the follicle, the follicular epithelium shrinks, folds and contracts forming a structure, the corpus luteum, resembling the resorption bodies in shape (Figs. 49 and 50). It is distinguished from the resorption bodies by its colourless appearance. Ovulation is of the Independent Successive type (Singh, 1954). Due to the rapid degeneration of the cells of the older corpora lutea, more than one corpus luteum was never found at the base of any ovariole. In the following two days, the rate of oviposition decreases (see chapter XI). A few basal oocytes appeared to be in the early stages of resorption, having become clear with the disappearance of yolk. Resorption bodies were found at the bases of 4 - 6 ovarioles by the end of the oviposition period. Very few living spermatozoa were found in the spermatheca during this period.

The post-oviposition period lasts for about two days. Egg development still continues and yolk is deposited at a slow rate. Resorption bodies were found at the bases of the ovarioles and the penultimate eggs appeared to be in various stages of resorption. At the end of this period each ovariole consisted of one oocyte. (Fig. 53). No traces of spermatozoa were found in the spermatheca, and the spermathecal gland became very small.

iv. Formation of resorption body

The formation of resorption body is illustrated in Fig. 54 The first sign of disintegration occurs in the oocyte contents. They contract and the yolk granules separate from the liquifying cytoplasm and become more conspicuous. In consequence, the vitelline membrane wrinkles (stage 1). All other signs of progressive degeneration are

manifested by the follicular epithelium. The cells increase in thickness from 6 to 20u; the nuclei become larger and peripherally located with vacuolated cytoplasm surrounding them (stage 2.). Then the cells project deeply into the oocyte, thus the vitelline membrane disintegrates and the contents of the oocyte and the follicular cells come in contact (stage 3.) The invading follicle cells break down in the central part of the oocyte and in section they appear as large separate cells (stage 4.). These have been called 'lecitholytic cells' by Lusis (1963), since their function appears to be similar to that of the vitellophages in embryonic development (Imms, 1957). Now the follicular cells absorb and phagocytose the liquified ovular substance and phagocytose the solid yolk elements. Throughout resorption and phagocytosis, the original contents of the oocyte disappear and the follicular cells rapidly fill up with yolk particles of different sizes (stage 5.). Then deformation in the follicular wall appears and continues until the entire ovular space is occupied by follicular cells or their extruded nuclei and cell contents. The follicle decreases in volume but the change in volume is not accompanied by any folding of the follicular wall, as in Locusta (Singh, 1954) and Schistocerca (Singh, 1958; Lusis, 1963).

The outer epithelial sheath which was greatly stretched over the mature oocyte and was discernible only with difficulty as occasional bulges, contracts and the nuclei become more easily seen lying closely together. Further degeneration of the follicular cells is seen in the disappearance of the chromatin content of their nuclei and the cells become extremely vacuolated. These vacuoles present a reticulate appearance in the central cytoplasm (stage 6.). Finally, the remaining components of the follicular cells undergo necrosis and absorption.

v. Formation of 'corpus luteum'

After the egg has been discharged from the follicle, the vollicular cells contract and collapse to form, finally, a shapeless mass of cells, the 'corpus luteum'. The process of corpus luteum formation is similar to that of the resorption body formation except that in the latter the process is a gradual one whereas the formation

of the corpus luteum progresses rapidly. Also, the formation of the corpus luteum is not accompanied by resorption of yolk; and finally, the epithelial plug always degenerates to allow for the passage of the eggs and therefore disappears before the formation of the corpus luteum, whereas in the formation of the resorption body, there is no preliminary disintegration of the epithelial plug.

b - The male reproductive system

i. Morphology (Fig 55).

The reproductive organs lie in the posterior half of the abdominal cavity, ventral to the alimentary canal. When fully mature, they extend anteriorly to the posterior margin of the fourth abdominal segment. The male reproductive system consists of two tubular testes, each composed of six elongate follicles held together in an extremely delicate membranous sheath. The testes, unlike th ovaries of the female, are maintained in position by the surrounding fat body and tracheae; there are no suspensory filaments. The six follicles narrow posteriorly to form six minute tubes, the vasa efferentia. The latter open into a long narrow tube, the vas deferens, corresponding to the lateral oviduct of the female. The two vasa deferentia are sinuous, extending posteriorly until they reach the eighth segment and then they pass anteriorly to open into the accessory glands. At the junction of the vasa deferentia with the vasa deferentia, on each side lies a small pouch-like structure, the vesicula seminalis. The accessory glands open in a crozier-shaped tube, the ductus ejaculatoris which leads to the aedeagus.

ii. Histology

The testicular tubules or follicles are enclosed in a common membranous sheath, the tunica. It is an extremely fragile ellipsoidal sheath and is clearly seen where it stretches between the apices of the tubules (Fig. 56). The tunica becomes more fragile as the male grows older. Each testicular tubule is invested in an epithelial wall, consisting of ellipsoidal cells. The epithelial walls between the adjacent tubules are rather compressed and their minute flattened nuclei

can be seen arranged in single rows (Fig. 56). Several authors, including Demandt (1912), Lomen (1914), Ruckes (1919), Korschelt (1924) and Snodgrass (1935), have suggested that the walls of the testicular tubules of insects serve as trophic intermediaries between the blood surrounding the testes and the germ cells as do the walls of the ovarioles. Conversely, Bonhag (1955) maintained that the walls of the tubules in Oncopeltus fasciatus do not seem to play any active role in the synthesis of nutritive substances for the germ cells.

The interior of each testis follicle is divided by thin noncellular membranes into an apical germarium and a series of cysts containing successive stages of developing germ cells (Fig. 56-59). The germ cells in each cyst are generally in the same stage of development; but in some cysts late anaphases of the first spermatocyte division and early stages of the second division are mixed together.

The germarium, as in the ovary, is the apical region of the testicular tubule containing the primordial sex cells - the spermatogonia. It is very small and hemispherical in shape and it can be overlooked in longitudinal sections cut obliquely. Among the psermatogonia, lies the apical cell, a characteristic feature of the testicular tubules (Fig. 57). Although the 'apical cell' or 'apical complex' as it is termed by Bonhag (1953) has been studied in many species belonging to different orders, in the Coleoptera it has been described only once, in the rove beetle, Staphylinus, by Holmgren (1901). The apical cell in T. granarium is therefore only the second example from the Coleoptera to be described in the literature. It consists of a mononuclear mass of cytoplasm which measures approximately 7 u in diameter, with darkly staining radiating strands. Different numbers of young spermatogonia surround the apical cell. As the testes grow older, the number of young spermatogonia decreases and the apical cells become no longer recognisable.

Spermatogonia, as they increase in size, arrange themselves in the form of a rosette. Each spermatogonial rosette soon becomes enclosed in a cellular envelope known as a sperm cyst. The spermatogonia in each cyst increase in size and apparently undergo repeated mitosis in developing into spermatocytes (Fig. 56).

The spermatocytes divide repeatedly forming the spermatids. Wigglesworth (1950) maintained that in most insects the first division

is a reduction division in which the chromosome number is halved. Then finally the spermatids develop into spermatozoa which occupy the basal half of the testicular tubule (Fig. 60.). The spermatozoa are not enclosed in cysts, but generally remain grouped in bundles as they were in cysts. A spermatozoon consists of a chromatin head and a long slender tail.

In the gaps between the membranes of the adjacent germ-cell cysts there are irregular cells, the trophocytes (Fig. 56.) which are associated with the spermatogonia, spermatocytes and spermatids. They contain large nuclei and coarse chromatin granules. Bonhag (1953) maintained that he could find these trophic cells in the region of the testicular tubule which is occupied by differentiated spermatozoa. In the present work, however, the trophic cells have never been found in the spermatozoal region. These cells are believed to serve as trophocytes for the developing germ cells in Euschistus sp. (Montgomery, 1910) and Oncopeltus (Bonhag, 1953).

The accessory glands (Figs. 55, 61 & 62a)

The wall of the glands consists of columnar binucleate cells with different thickness. The wall is covered externally by a very thin nucleated sheath. A refractile inner border, intima, lines the lumen of the glands. The binucleate epithelial cells have homogeneous cytoplasm, but in active glands there are, interspersed, swollen cells which contain larger vacuoles. The interior of the gland is filled with a colourless, cloudy fluid that forms a granular or spongy coagulum in sections and stains dirty blue with iron haematoxylin.

The vesicular seminalis (Fig. 62b)

The wall of the vesicula seminalis consists of small cuboidal epithelial cells and is continuous with the epithelial lining of the vasa deferentia. The outer surface of the vesicular seminalis is covered by a thin sheath with ovoid cells. The vesicular seminalis contains large masses of spermatozoa.

iii. Quantitative and qualitative changes of male reproductive organs

As in the female, maturation of males in the presence of females was completed in a shorter time than that of isolated males. The former leave the larval skin after 24 hours from their emergence whereas isolated males remain in the larval skin for 36 hours after emergence. The reproductive system of the male changes very quickly. The histological changes in the male reproductive system, however, are not as spectacular as those in the female organs.

Measurements were made of the testicular tubules, on the width of the accessory glands and on the length of the vesiculae seminales. The latter were also examined for the presence of spermatozoa. The observations are tabulated and shown in Table 6.

Table 6. Morphometric measurements of the male reproductive organs based on examination of 5 adults of each stage.

Physiological stages	Length of testis in u.	Width of accessory gland (widest part)	Length of vesicula seminalis (V.S)	Condition of V.S.
Beginning of maturation (0-3 hrs. old)	450	90	85	empty
End of maturation	700	180	150	full
Unmated male	$700 \pm$	$180 \pm$	$150 \pm$	packed
Mated male	900	250	200	full $\pm$

There was conspicuous growth in the reproductive organs during the maturation period which is 24 - 30 hours from the time of eclosion till the males leave the cast larval skin. In unmated males, no further growth in the reproductive organs occurred and their size was maintained with small fluctuations for about six days and then decreased rapidly. In males reared with females, the growth continued and the testes developed twice the length of those in the immature males.

The accessory glands the vesiculae seminales were three times as large as those in the newly emerged adults.

Discussion

Examination of the ovarioles in females reared in isolation and with males, revealed that egg development and maturation took place in both, but at a slower rate in the isolated females. The latter leave the larval skin after maturation is completed and their ovaries developed to the shape and size shown in Fig. 42 within two days from the emergence of the imago; whilst in females reared with males, maturation was completed within a period of 27 - 30 hours after which they leave the cast larval skin. Their ovaries resembled those in Fig. 42. Similarly, maturation and ovarian development start sooner in mated S. gregaria than in isolated adults (Norris, 1954; Highnam and Lusis, 1962).

In the absence of the male the development of the terminal oocytes proceeds, but all the oocytes on reaching maturity are resorbed instead of being ovulated and resorption bodies are found. In mated females, the egg development proceeds rapidly in the first few days and all the eggs are ovulated and corpora lutea are found at the bases of the ovarioles. Later, however, oviposition proceeds at a slower rate and a few occytes are resorbed instead of being ovulated. Resorption bodies are therefore found in both mated and unmated females of T. granarium, but a higher percentage in the latter. This is in agreement with the findings of Singh (1954) in Locusta and Schistocerca and of Highnam and Lusis (1962) in Schistocerca. In Dermestes, however, Ladduwahetti (1962) maintained that 'resorption bodies' never occur in the mated females and are formed only in the unmated ones.

In T. granarium, the resorption body could always be distinguished in histological preparations from the corpus luteum by the former being pigmented. In Schistocerca and Locusta, corpus luteum could be seen pigmented after ovulation of subnormal eggs (Singh, 1958). In T. granarium, the decrease in volume of the degenerating follicle is not accompanied by any folding of the follicular wall. Instead, there is an extrusion of some cells and cell contents into the oocyte area. Similarly, the follicular epithelium does not fold in the degenerating

follicle in Locusta and Schistocerca (Singh, 1958). Conversely, Lusis (1963) maintained that the decrease in volume of the degenerating follicle is accompanied by large folds in the tunica propria and the follicular epithelium.

In T. granarium, ovulation is of an independent successive type, so that each corpus luteum arises by degeneration of a single follicle and a simple corpus luteum was only found at the base of an ovariole. Compound corpora lutea as in Dysdercus and Ephestia (Singh, 1958) have never been found.

The spermathecal gland enlarged progressively. However, in unmated females the gland was smaller than in mated females and was largest just before oviposition.

The histological structure of each ovariole is in agreement with the findings of many other investigators in different insects. No distinct stratification among the trophocytes in the germarium, however, could be observed as in Hemiptera (Bonhag, 1955a, b; Bonhag & Wick, 1953; Johannson, 1958). The nutritive cords were seen in all the specimens examined, in newly emerged adults, attached to the developing oocytes and passing from one follicle to another to penetrate the inter-follicular cell masses. There is, however, no distinct trophic core as in Hemiptera (Bonhag, 1955). Nutritive cords disappear after oviposition. The characteristic change and the transition of the epithelium surrounding the developing oocytes, from columnar to a cuboidal and to a flattened type seem designed to accommodate changes in volume of the growing oocyte. Mitotic figures were not discernible in the columnar and cuboidal epithelia of T. granarium as reported on Oncopeltus fasciatus (Bonhag, 1953).

Maturation was achieved earlier also in males of T. granarium when they were reared with the female. Spermatogensis proceeded at a slower rate in isolated males. Their reproductive organs reached a maximum size, before copulation, in a 36 - hour period whereas in males reared with females the reproductive organs developed to the same size in a 24 - hour period.

The 'apical cell' has been found in the male of T. granarium. Similarly, it was found in many insects of different orders (see Carson, 1945). Conversely, the 'apical cell' has not been found in testicular tubes of D. melanogaster (Demerec, 1950). The 'apical cell' in T. granarium is mononucleated whereas in Oncopeltus it is multi-

nucleate (Bonhag, 1953). The histological appearance and position of the apical cell in the testis follicle of T. granarium indicate that it is a trophic tissue and may be analogous to the apical trophocytes of the ovariole. Previous investigators came to the conclusion that the apical cells are a nutritive and supporting element for the developing germ cells - see Carson, (1945).

As in most insects, the interior of each testis follicle of T. granarium is divided into a series of cysts containing successive stages of developing germ cells. Conversely, Demerec (1950) maintained that a distinct zonation into successive developmental regions is not evident in the testis follicle of D. melanogaster.

2. The neurosecretory system in adult T. granarium

a - Morphology and anatomy

As in the larva and pupa, the neurosecretory system of adult T. granarium is located in the head. No neurosecretory cells are found in thoracic or the abdominal ganglia. The frontal ganglion is also devoid of neurosecretory cells.

No differences in the arrangement or in the components of the neurosecretory system between male and female have been found apart from the slightly larger size of the neurosecretory system in the female.

The neuro-endocrine system of T. granarium is depicted in Figs. 63 & 67. It consists of the brain, occupying a large part of the head capsule and lying above the oesophagous. It is linked to the suboesophageal ganglion by two para-oesophageal connectives. Two nerves arise postero-ventrally from the protocerebrum, which connect the brain to the corpus cardiacum. These nerves are known as the external and internal nervi corpus cardiaci of which the latter is the wider. These two pairs of nerves which are responsible for innervating the corpora cardiaca occur in all pterygote insects (Cazal, 1948). The corpora cardiaca are roughly cone-shaped; and their distal ends are joined to the two subspherical corpora allata. Cardiaco-allatum nerves could not be found in T. granarium. However, a very fine nerve was detected

connecting the corpus cardiacum/allatum to the suboesophageal ganglion. The latter lies ventrally to the oesophagus and is connected to the rest of the ventral ganglia by two wide connectives.

b - Histology

i. The brain

The brain is invested by a thin connective tissue sheath which consists of two layers, the outer non-cellular neural lamella and an inner neural sheath. Within these layers the brain can be divided into two zones: the cortical zone, which comprises neurosecretory cells, glial cells, globule cells and ordinary motor neurones; and the medullary zone consisting of the neuropile which is principally made up of axons.

The neurosecretory cells are divided into four groups, two medial groups lying close together in the mid-dorsal region of the pars-intercerebralis of the photocerebrum and two lateral groups, one in each cerebral hemisphere, and located dorso-laterally just outside the pars-intercerebralis region.

The medial groups (Figs. 64 & 67)

Each group is lodged deeply in each hemisphere and lies directly under the neural sheath. In cross section, there is no distinctive demarcation of the two groups and they often appear as one medial group. Each group consists of 12 A-cells, containing varying amounts of paraldehyde-fuchsin-stained granules, and 2 - 4 B-cells distributed between the A-cells. The histological changes of these cells at different stages of the life cycle are presented in page

The lateral groups (Figs. 65 & 67)

They lie in the cortical zone anteriorly to the medial groups. Each group consists of two A-cells. Their staining affinity and the histological details of the cytoplasmic inclusions suggest that the A-cells are similar to A-cells of the medial group though slightly smaller. No B-cells could be found in the lateral groups.

ii. The suboesophageal ganglion

The suboesophageal ganglion is invested by a thin connective tissue sheath which consists of two layers, an outer neral lamella which is non-cellular and a homogeneous inner neural sheath which is a one cell layer with very minute nuclei. The body of the ganglion is divided into two regions, a large medulla or neuropile and a thinner outer cortex containing the neurones. The cortex is generally thicker on the ventral than on the dorsal side.

Neurosecretory cells in suboesophageal ganglion (Fig. 66)

One single A-cell (comparable to those of the medial groups in the brain) could be found in the mid-ventral line of the ganglion. It lies deep and close to the medullary zone. No C - cells were observed.

iii. Neurosecretory pathways

The pathways traversed by the axons of the A - cells in the brain could be easily traced in the medullary zone owing to the presence of a deep purple stained secretory material carried along them. The pathways traversed by the B - cells are distinct until they enter the neuropile and then they become masked by the A - cell secretions. As has been shown in the larvae, the axons from the two medial groups pass anteriorly into the neuropile and cross each other, continuing ventrally and finally passing into the internal nervi corpus cardiaci. Thus each corpus cardiacum is innervated by axons from the opposing medial group of the neurosecretory cells (Fig. 67).

The axons from each lateral group of neurosecretory cells run directly into the neuropileand ultimately enter the external nervi corpora cardiaci of their own side. The axons of the lateral groups, immediately before they leave the brain, are intimately close to the pathways of the medial groups (Fig. 67).

The neurosecretory pathways of the sub-oesophageal ganglion could not be traced successfully. The axon of the A - cell runs deeply into the neuropile, beyond which it cannot be traced. It may be suggested that the neurosecretory material carried along this

axon is distributed to the nerves arising from the ganglion.

iv. The corpora cardiaca (see Fig. 68)

The corpus cardiacum is of nervous origin (Hanstrom, 1940), but neurones are uncommon in the functional organ. In adult T. granarium the gland is roughly cone-shaped, its distal base being joined to a compact, spherical corpus allatum. Histologically, the gland, as in the larva and pupa, consists of two types of cells embedded in a matrix; these are the 'chromophile cells' and the 'chromophobe cells' described by Cazal (1948). The location of these cells in the gland, their histological structure and their tinctorial affinity are exactly the same as in the larva and pupa.

Among these two types of cells, fine axons from the neurosecretory cells of the brain and droplets of stained secretory material could be found in the matrix of the corpus cardiacum. From the histological studies of the corpus cardiacum at different stages, the gland seems to act as a storage organ and is involved in the release of the brain hormone.

v. The corpora allata (see Fig. 68)

Each corpus allatum is a compact body invested by a thin non-cellular membrane, which is continuous with that of the corpus cardiacum. The inclusions and the structure of this gland vary from one physiological stage to the other. It consists of cells whose outlines can be either easily seen, as in newly emerged adults and active glands or discerned only with difficulty as in older and inactive adults. The nuclei are spherical or subspherical in shape. They are either closely packed together and distributed quite homogeneously, or they are widely spaced and the intercellular areas are clear. The activity of the gland is accompanied by an increase in volume by means of the cell and nuclear increase only. No mitotic activity has been discerned. Neurosecretory products from the brain could not be found in the gland. However, some of the axons of NCCI and NCCII which innervate the corpus cardiacum traverse their entire lengths into the corpus allatum, being dilated locally with globules of neurosecretory material. These axons may be

considered to constitute the cardiaco-allatum nerve which is not distinct in adult T. granarium. Some preparations, however, show that the corpora cardiaca and allata are connected by two laterial strands of tissue, giving the impression of nerves (Fig. 68.); but these are artifacts due to shrinkage of both glands.

c - The histological changes of the neurosecretory system during adult life history

To compare the activity of the neurosecretory system at different phases, the neurosecretory cells were divided into four arbitrary categories designated as stages I to IV. A cell in stage I had little neurosecretory material whereas cells in stage IV were packed with secretions.

The relative quantities of neurosecretory cells' secretion, discharged along the axons and that which was stored in the corpora cardiaca at various stages, were represented by arithmetical symbols from $\pm$, + , ++ , and +++

Results

The Female

The histological and morphological changes of the neurosecretory system in female T. granarium are summarized in Table 7.

i. Changes in neurosecretory cells of the brain.

In the white adult, 0 - 3 hour old female, the majority of the A - cells are spherical in shape with a small axon hillock which gives the cell an onion-like shape (Fig. 69.). The cells contain minute secretory particles which associate to form aggregates or granules of varying size. Vacuoles could be traced. Very few traces of the A - cells' secretion could be traced along the pathways to the corpora cardiaca. The volume of the cells gradually increases and the axons along the pathways become more obvious as they carry increasing amounts

TABLE 7. The histological and morphometrical changes of the neurosecretary system in female *T. grenarium* at different stages, based on 5 adults per stage.

Physiological phase	Volume of cells		Stage of cells		A-cells secretion along the axons	A-cells storage in corpus cardiacum	Volume of corpus cardiacum	Volume of corpus allatum
	A	B	A	B				
Beginning of maturation (0-3 hours old)	310	297	II	II	+	+	24990	18714
Maturation	422	322	IV	III	++	++	41650	46785
Copulation	409	322	III	II	++	++	41340	46800
Pre-oviposition	498	380	III	III	+++	+++	62470	74850
Oviposition	569	398	II	I	+++	±	98760	168420
Post-oviposition	390	270	I	I	±	±	12390	12100

of secretory materials. At the end of the maturation period, immediately after the adult leaves the larval skin, the cells are pyriform and contain large quantities of small granules and aggregates which fill most of the perikaryon. Vacuoles have not been observed and the axons along the pathways increase (Fig. 70).

In unmated females, this condition is, more or less, maintained for two to three days and then the cells gradually become smaller and vacuolated. The neurosecretory material along the pathways and in the corpora cardiaca decreases.

In mated females, during copulation, the cells were full of secretion and no change was observed in the amount of secretory products along the axons. Within the following 24 hours (the pre-oviposition period) varying amounts of inclusions were found in the cell bodies, where the cells are large the inclusions are also large and completely fill the cell-bodies. The axons carried varying amounts of secretory material and were rather prominent (Figs. 71 and 72). In the following 3 - 4 days, the oviposition period, the cells contained small amounts of secretion and the axons were richly packed with secretory material, running deeply into the neuropile and entering the corpora cardiaca (Figs. 73 and 74).

In the following one to two days, the post-oviposition period, the cells were vacuolated and contained very few inclusions which aggregated around the vacuoles or adhered to the cell wall. There was a conspicuous decrease in the amount of the secretory products along the axons, which were discernible only with difficulty (Fig. 75).

The quantity of A-cell secretion stored in the corpus cardiacum was rather small in the 0 - 3 hour-old adult. The amount increased during maturation and reached its peak in the pre-oviposition period. At the phase of oviposition, the amount of A-cell secretion stored in the corpus cardiacum was small, although the neurosecretory cells were at a high level of secretory activity.

These observations suggest a differential rate of release of secretion from the cells, transportation along the axons and discharge from the corpus cardiacum, at different phases.

No marked changes, compared to those in A-cells, could be observed in the B-cells during maturation and oviposition. At the phase of maturation, the cells contained increasing amounts of secretion stained green-blue with PF. This amount of secretion was maintained

with little fluctuation throughout the adult life.

The amount of B - cell secretion stored in the corpus cardiacum followed a similar pattern, varying slightly with the activity and appearance of the B - cells.

ii. Changes in neurosecretory cells in the suboesophageal ganglion

The conspicuous changes occurring in the brain A - cells during the different physiological phases were paralleled in the A - cell of the suboesophageal ganglion. During maturation, increasing amount of secretory product accumulated in the cell, accompanied by an increase in the cell volume. Coinciding with oviposition, the cell contained little secretory product and the axons became full of product. This suggests a high level of secretory activity which was maintained with minor fluctuations throughout the oviposition phase. During post-oviposition period, the cell volume decreased and the axons could no longer be recognised.

Unfortunately it has not been possible to trace the entire axonal pathways of this call. As in the larva and the pupa, it is reasonable to assume that the neurosecretory products of the ganglion are discharged along the nerves arising from the ganglion. This assumption agrees with the findings of Delphin (1963) in Schistocerca gregaria. This will be discussed in the general discussion (see Chapter XIIX)

iii. The qualitative and quantitative changes in the corpora cardiaca

The volume of the corpus cardiacum increased gradually to a maximum during maturation. This volume was then maintained, with fluctuation, during the whole life of the unmated female. No spectacular changes in the volume and the distribution of the gland cells were observed. In mated females, the volume of the gland increased steadily and was greatest during oviposition (Fig. 74.). Just after oviposition, the gland decreased considerably in volume and was smallest at the end of the post-oviposition period (Fig. 76.). There appears, therefore, a correlation between glandular activity of the corpus cardiacum and

oviposition.

The secretory 'chromophile cells' enlarged correspondingly with the size of the gland at the phases of ovarial maturation and oviposition. They became ill-defined at the end of the post-ovisposition period.

iv. The qualitative and quantitative changes in the corpora allata

The sequence of changes in the corpus allatum during adult life were no less spectacular then the changes in the corpus cardiacum. During maturation the volume of the gland increased steadily. It consisted of compactly arranged cells and no vacuoles could be seen. This volume and distribution of the gland-cells were maintained with slight fluctuation throughout the life of the unmated female. In mated females, however, the corpus allatum showed signs of activity at different physiological phases suggesting that a great role was played by this gland in reproduction. During the pre-oviposition period, the gland conspicuously increased in size as a result of a marked increase in the cells. No mitotic figures were seen at any stage. In the oviposition period, the volume of the gland was greatest and this size was maintained throughout the period of oviposition (Fig. 74.). The cells of the central region were more closely packed, whereas those in the periphery were loosely arranged with large inter-cellular spaces and distinct cell walls. Just after the oviposition, the size of the gland and its cells decreased and was smallest at the end of the post-oviposition period (Fig. 76.).

The Male

The qualitative and quantitative changes of the neurosecretory system in the male at different stages are summarized in Table 8.

i. Changes in the neurosecretory cells of the brain

In 0 - 3 hour old males, the cells were more or less spherical with a very short hillock (Fig. 77). They contained varying amounts of secretory products which filled most of the perikaryon. Few vacuoles were found. The cells gradually increased in size and accumulation of neurosecretion also increased. Few secretory products appeared along the pathways. At the end of the maturation period, the cells were packed with neurosecretion and the axons were clearer along the pathways. The condition was maintained with small fluctuation throughout the life of the unmated male. In the mated male, however, during copulation, the amount of secretory products in the cells conspicuously decreased and a large amount of secretions were seen along the pathways (Fig. 78). After copulation, there was a tendency for the secretory products to accumulate in the cells and to decrease along the pathways. This pattern was observed for three to four days after which the cells were senescent and the axons were no longer recognizable along the pathways.

The amount of brain secretory product stored in the corpus cardiacum fluctuated throughout the male life. It was smallest during copulation, suggesting a regulating role played by the gland for the brain secretion at different stages.

ii. Changes in the neurosecretory cell of the suboesophageal ganglion

The histological changes in the A - cell of this ganglion follow the same pattern of those in the brain. As in the female, it has not been possible to trace the entire axonal pathways of this cell.

iii. The qualitative and quantitative changes in the corpora cardiaca

There was an initial increase in the volume of the gland

TABLE 8. The qualitative and quantitative changes of the neurosecretary system in the male at different stages, based on 5 adults per stage.

Physiological phase	Volume of cells (u^3)		Stage of cells		A-cells secretion along the axons	A-cells storage in corpus cardiacum	Volume of corpus cardiacum	Volume of corpus allatum
	A	B	A	B				
Beginning of maturation (0-3 hours old)	280	210	II	II	+	+	21600±	15700±
End of maturation	389	290	IV	II	++	+++	39000±	29870±
Copulation	383	278	II	II	++	+	39000±	29800±

during maturation. This volume was then maintained with small fluctuations throughout the male life. The gland was smallest in old males and largest just after the maturation phase.

iv. The qualitative and quantitative changes in the corpora allata

Although the rate of histological differentiation in the corpus allatum shows individual variation, examination revealed that corpora allata are larger in mated males than in unmated males, suggesting that the gland plays a role in controlling reproduction.

Discussion

The foregoing results revealed considerable evidence for a relationship between reproduction and the neurosecretory system in adult T. granarium. The following few lines are an attempt to interpret the histological observations especially in regard to expressing, in terms of 'activity' and 'inactivity', the amounts of neurosecretory material present in the neurosecretory system at any one time.

Some investigators of neurosecretion have considered that A - cells are more active when they are full of inclusions (Dupont-Raabe, 1952, Arvy and Gabe, 1952, Formigoni, 1956, Herlant, Meewis and Paquet, 1956). On the other hand, Highnam (1961a) stated that the amount of material contained in a neurosecretory cell atany timedepends both upon its rate ofsynthesis and its rate of discharge. He also maintained that in a neurosecretory system containing small amounts of material, the material is discharged as soon as it is formed and therefore the system as a whole is 'active'. He was able to substantiate this by his use of radioactively labelled cystine which was taken up more rapidly in the neurosecretory system having a few inclusions than in a system packed with secretions. Ladduwahetty (1962) preferred to use the term 'release' instead of 'active'. She considered the neurosecretory system to be in a state of 'high release' when the A - cells contain few aggregates of secretion and the axons and the endocrine organs contain large amounts of material.

In Galeruca tanaceti, the criterion for assessing 'activity'

by histological observations of the neurosecretory cells were unsatisfactory as the insects at any stage of the life cycle have neurosecretory cells which contain varying amounts of inclusions and varying nuclear volumes (Siew, 1963). Siew, however, assumed that a neurosecretory cell at its initial cycle of activity contains no secretion or very little. He supported his observation by the use of S^{35}-D-L cystine, which was taken up more rapidly in the neurosecretory cells containing few inclusions than in those packed with inclusions.

In T. granarium, it seems reasonable to consider both rate of 'synthesis' and rate of 'release'. Consequently, the terms 'active' or 'inactive' have been used for describing the rate of 'synthesis'. The terms 'low' or 'high' release are used for describing the rate of the 'discharge' or 'release'. It has been shown in diapausing larvae kept with food that the neurosecretory cells were packed with inclusions but the axons lack or contained very little secretory products. In this case the cells are considered to be 'active' in synthetizing neurosecretory material but in a state of 'low release'. Conversely, when the dormant larvae were kept without food, there was a small quantity of inclusions within the neurosecretory cells and along the axons. Thus the cells are considered to be 'inactive' in synthetizing the secretory product and at 'low' rate of release. In non-dormant larvae, where physiological processes proceed progressively, the neurosecretory cells are considered to be both 'active' and at 'high' rate of release.

Changes in activity and release can also be recognised in the neurosecretory system of the adult during the phase of maturation, copulation, pre-oviposition, oviposition and post-oviposition.

During the phase of ovarial and testicular maturation, the neurosecretory cells were 'actively' synthetizing the secretory products and the rate of synthesis was higher than the rate of release. Thus the cells were filled with neurosecretion. This is also apparent in the neurosecretory system of isolated females and males, as their neurosecretory cells contained large amounts of material and the axons contained few inclusions.

During copulation, the cells appear to be still 'active' in synthetizing the neurosecretory material and at 'low' rate of release in the female but at 'high' rate of release in the male. Thus the cells

in the female were filled with neurosecretion and the axons contained little secretory material, whereas in the male the cells contained smaller inclusions and the axons were loaded with secretory products.

During the phase of pre-oviposition in the female, and in the mature mated male, amounts of secretory material increased in the axons and in the corpora cardiaca, but the neurosecretory cells remained full of inclusions. This suggests that the cells are 'active' and the rate of release is equal to the rate of synthesis. A comparable condition is seen in Schistocerca reared with mature males during the phase of ovarial development (Highnam, 1961, 1962) and during the phase of oviposition in G. tanaceti (Siew, 1963).

During the phase of oviposition the neurosecretory cells of the female were also synthetically 'active' and in a state of 'high' release. However, in this case the rate of release was much higher than the rate of synthesis, so consequently the cells contained very few inclusions and the axons contained large amounts of secretion. This is also comparable with the findings of Highnam (1961, 1962) in S. gregaria.

During the phase of post-oviposition and in the old males both rate of synthesis and rate of release decreased. Finally, the neurosecretory cells became senescent and consequently 'inactive' synthetically. The whole system was then in a state of 'low' release. Other examples of 'inactive' neurosecretory cells will be described when larvae treated with insecticides are considered (see Chapter XII.)

The above interpretations are set forth in Fig. 79 which illustrates the correlation of synthesis and release of neurosecretory substance with different physiological phases.

X. EXPERIMENTAL INVESTIGATIONS INTO THE ROLE OF THE NEUROSECRETORY SYSTEM IN CONTROLLING OVARIAL AND TESTICULAR MATURATION AND OVIPOSITION

Introduction

The preceeding histological studies have demonstrated that considerable changes occur in the neurosecretory system which seem to exert a regulating influence on the reproductive system in both female and male. However, histological studies by themselves are not sufficiently conclusive, and final experimental proof is needed to ascertain the individual roles played by the components of the neurosecretory system in controlling ovarial development, testicular maturation and oviposition. Also it was found desirable to investigate the relationships between the various components.

Where the experiments involved removal of any part of the neurosecretory system, cauterisation was considered preferable to extirpation which was more difficult in such a small insect. However, the corpora cardiaca and allata of T. granarium were not satisfactorily cauterised. Therefore, the study was divided into two sections: 1. neurosecretory cells in the brain and the suboesophageal ganglion were cauterised and the overall effects on the reproductive system and other parts of the neurosecretory system were studied: and 2. 'Farnesol' was applied to individual insects to demonstrate the probable role of the corpus allatum hormone in ovarial and testicular development.

Implantation experiments were attempted following the method used for the larva but failed to give satisfactory results.

1. Cauterisation experiments

The first attempts at cauterisation were unsuccessful and mortality was almost 100%. The mortality was caused either through handling or by the operation itself. With practice, the operation yielded 70 ± 90% survival and the damage was mostly caused through handling.

Twenty mature unmated females, forty mated females after

showing the first sign of oviposition and twenty mature males were used. Half of their numbers had the brain neurosecretory cells cauterised and the rest had their suboesophageal neurosecretory cell cauterised.

Results

The results are summarised in Tables 9 and 10.

a. Cauterisation of the neurosecretory cells in the brain

i. Unmated females

Immediately after the operation the cauterised females remained motionless, but after 30 minutes to an hour, they recovered from the operation. However, all the females had their genitalia protruding behind and they were kept out until the females were dissected, 4 - 6 days after the operation. Also, some of them showed abnormality in their behaviour, e.g., they tended to wander around with open elytra and outspread wings which were occasionally seen to vibrate. When males were introduced to them, the males were always rejected and copulation never took place. By the end of the fifth day after the operation their distended abdomens were contracted, having a flat appearance. Adult T. granarium do not normally feed and the cauterised females did not feed, but physiological processes connected with digestion and excretion apparently proceeded normally for large numbers of compact faecal pellets were found, as in uncauterised females.

Dissection revealed that brain cautery not only inhibits egg development in T. granarium, but it also caused the ovarioles to atrophy (Fig. 80). The ovarioles lost their attachment to the body wall and each ovariole became a crozier-like structure with a bulb-shaped germarium.

In the germarium, differentiation of the trophocytes was no longer discernible and they were very small compared to those in control females. The oogonia and the young oocytes disappeared and no trace of nutritive cords could be discerned (Fig. 81). In the vitellarium, degeneration and absorption of oocytes rapidly occurred in all parts of

the vitellarium. This result differs from the resorption in ovarioles of females reared without males, where it usually starts in the terminal oocytes followed by the penultimate ones. Being cut off from the nutritive cells, the small growing oocytes degenerated, died and were absorbed by their follicular cells. In the penultimate oocyptes where no yolk was formed before resorption, the follicular epithelium showed the earliest sign of degeneration. The oocyte contents were vacuolated and gradually contracted and absorbed by their follicular cells. The process of degeneration of the terminal oocytes, where yolk is present, took place in the same way as it did in females reared without males (see Page 75 and Fig. 54).

The processes of degeneration and absorption proceeded until the vitellarium became a thread-like structure consisting of degenerated follicular cells and few bulges of degenerating oocytes (Fig. 81).

Examination of the other parts of the reproductive system revealed atrophy of the spermatheca and spermathecal gland.

Examination of the head region revealed that the neurosecretory cell in the suboesophageal ganglion in the first 24 hours, contained very little secretion and decreased in size. It then gradually diminished and by the end of the third day it was no longer recognised. The secretory products from the brain in the corpora cardiaca completely disappeared. Corpora cardiaca and allata were greatly shrunken.

The control females also had their genitalia protruding for almost one hour after the operation. Then they could operate them normally and when males were introduced they copulated and laid eggs. Dissection revealed well-developed ovaries with fully mature basal oocytes.

ii. Mated females

A great care was taken in handling these insects as their abdomens were fully expanded and a slight over pressing would cause them to burst. Nevertheless, 50% of the operated animals escaped damage in handling.

Four females laid one egg each within the first four

hours following the operation and then they completely ceased oviposition. The rest did not oviposit at all throughout the post-operative period. Females dissected within the first 24 hours after the operation contained little yolk in their maturing oocytes and different grades of resorption occurred in the terminal and penultimate oocytes. By the end of the fourth day after the operation, their abdomens contracted, resembling those of the cauterised unmated females and dissection revealed a complete atrophy of the ovaries. The spermathecal gland and the spermatheca were shrunken and no spermatozoa were found in the spermatheca.

Sections of the head region revealed that the neurosecretory cell in the suboesophageal ganglion became 'inactive' and at 'low' rate of release. Brain neurosecretion disappeared completely from the corpora cardiaca, which were greatly shrunken. The corpora allata were also shrunken.

iii. Mature males

The insects remained motionless for about one hour after cauterisation. Then they appeared to recover completely from the operation and started walking slowly. None showed any abnormality in behaviour except in not being able to mount the female. This was, presumably due to the exhaustion resulting from the operation and the heaviness of the minute head caused by the presence of the drop of the 'Durofix' used for sealing the wound. However, when helped to mount the female, they soon dismounted and copulation did not take place in spite of the activities of the female's genitalia. Other physiological processes, e.g., feeding, digestion and excretion proceeded normally. The male genitalia did not protrude but remained in place.

Dissection revealed that in the majority of the cauterised males the vasa deferentia and the ejaculatory duct lost their characteristic coiling and crozier-shape features and stretched anteriorly so that the testes were found in the thoracic cavity and the accessory glands were in the anterior half of the abdomen. Also, the vasa efferentia became conspicuously elongated and easier to be seen whereas in normal males they were so small that they can be overlooked (Fig. 82). The testes were in an atrophied state and the germarium atrophied

completely so that only a very few testicular tubules showed the germarium. In these germaria, the apical cells were never seen. Examination of the vesicula seminalis, which became shrunken, revealed the absence of spermatozoa. The accessory glands shrank and their wall cells were in a state of collapse (Figs 82 & 83)

Examination of the head region revealed that the corpora cardiaca and allata were greatly shrunken and the brain neurosecretion completely disappeared from the corpora cardiaca.

Control males could not mount the females easily. When helped to do so, however, they remained mounting the females and copulated. Examination of the females revealed the presence of the spermatozoa in both bursa copulatrix and spermatheca.

b. Cauterisation of the neurosecretory cell in the suboesophageal ganglion.

i. Unmated females

About 60% of the operated females survived the operation. Digestion and excretion processes proceeded normally. Their behaviour was also quite normal but their abdomens became slightly contracted. If their genitalia were protruded they soon withdrew them. When males were introduced to them two only copulated. Examination revealed the presence of spermatozoa in the bursa copulatrix and the spermatheca. Different grades of resorption occurred in the terminal, penultimate and antepenultimate oocytes (Fig. 84). Oogonia and young oocytes were present but in smaller numbers than in control females. Egg development apparently proceeded at slow rate and yolk deposition ceased.

Examination of the remaining components of the neurosecretory system revealed that the brain neurosecretory cells, after 24 hours, were in a condition comparable to that of the cells in 'low release'. The corpora cardiaca and allata were shrunken.

In the control females, behaviour and oviposition proceeded normally.

ii. Mated females

All survivors laid one egg each within the first few hours after the operation and then all ceased oviposition Dissection revealed exactly the same findings as in cauterised unmated females.

Control females laid eggs.

iii. Mature males

No abnormality could be observed in the behaviour of the operated males. Dissection revealed that testes decreased in size and the number of cysts was less than normal. Spermatogenesis apparently proceeded at a slow rate but no spermatozoa were found in the vesicula seminalis, which became slightly shrunken. The accessory glands were also slightly shrunken and secretion in the lumen was wanting.

Examination of the head region revealed a shrunken corpus cardiacum and allatum.

Control males copulated and had their vesicular seminales full of spermatozoa.

2. Farnesol experiments

From the results obtained after cauterisation of the neurosecretory cell in the suboesophageal ganglion, it is evident that the ganglion does not interfere with sexual behaviour. Females deprived of their suboesophageal ganglion secretory cell can still be stimulated by the presence of the male and can still copulate. Also, its hormone does not initiate oocyte differentiation and does not inhibit spermatogenesis. Yet it seems to be necessary for the brain/corpora cardiaca/allata to function at a level suitable for oviposition, spermatogenesis, yolk deposition and the activity of the accessory gland. Two questions arose from these studies: 1. does the neurosecretory cell in this ganglion act to influence the secretory activity of the brain and corpora cardiaca/allata, restricting such activity to a level suitable for oviposition, spermatogenesis and the production of secretion in the

accessory glands; or 2. does it actually contribute a hormone necessary for these processes to proceed ?

In order to decide between these possibilities, two kinds of experiments were employed: 1. cauterisation of the neurosecretory cell followed by implantation of suboesophageal ganglia or corpora cardiaca/allata; and 2. cauterisation of the neurosecretory cell followed by treatment with farnesol as a 'yolk forming hormone' and as an 'accessory gland activator'.

Unfortunately no results were obtained from undertaking implantation experiments. Some insects died while the ganglion was being implanted in the abdomens and in the most nearly successful experiments, death occurred after six hours. Therefore this experiment was abandoned.

Materials and technique

Ten ovipositing females and ten mature males were used. Their suboesophageal ganglion was cauterised and they were left for three hours to recover completely. Undiluted farnesol was then spread with the round head of an entomological pin over the body twice a day.

For controls, five ovipositing females and five mature males had their suboesophageal ganglion cauterised and they were not smeared with farnesol.

All survivors were fixed four days after the operation.

Results

The results are summarised in Tables 9 and 10.

i. Ovipositing females

None of the treated and control females had deposited eggs. Egg development proceeded at a slow rate. In treated females, however, yolk had been deposited whereas in control females yolk deposition was completely inhibited. Resorption occurred in both treated and control females but the percentage resorption was considerably lower in treated females. Each ovariole in each treated female

contained 5 - 6 developing oocytes whereas in control females an ovariole contained 3 developing eggs. These are compared to a normal ovipositing female where each ovariole at that time contains 9 - 10 developing oocytes.

No difference in the other parts of the reproductive system in both treated and control females has been discerned.

Examination of the head region revealed that the neurosecretory cells in the brain in the treated and control females were at low rate of release. Corpora cardiaca and allata were also shrunken.

ii. Mated males

No spectacular differences between treated and control males, compared with those of the females, were found. The only change in the reproductive system of the treated male was that the accessory glands became filled with secretion.

Discussion

The preceeding observations indicate that the neurosecretory cells of the brain exert an overall controlling influence on maturation, development of ovaries, spermatogenesis and sexual behaviour in the adult T. granarium. Females and males, deprived of their brain neurosecretory cells cannot copulate and their ovarial development and spermatogenesis are completely inhibited. The atrophy of the ovarioles may be due to a failure of protein synthesis. Failure of egg development has been suggested to be due to a failure of protein synthesis in Calliphora after extirpation of the median neurosecretory cells of the brain (Thomsen, 1952) and in Rhodnius after allatectomy (Wigglesworth, 1954). A neurosecretory control of protein metabolism has been demonstrated in Periplaneta (Bodenstein, 1953) after extirpation and implantation of corpora cardiaca and in Schistocerca (Hill, 1962) after removal and implantation of the brain or corpora cardiaca. Failure of egg development has also been shown to be due to a failure of protein synthesis after cauterisation of the brain neurosecretory cells or extirpation of corpora cardiaca in Schistocerca (Highnam et al, 1963). Cauterisation of neurosecretory cells of the brain caused atrophy to

TABLE 9. Morphometric measurements of the reproductive organs and their conditions in operated mated females of *T. granarium* based on all survivals, four days after the operation.

Part of N.S. cauterized	No. of adults	No. of survivals	Germarium diameters in u	Largest trophocyte diameters	Ovarial condition	Vittellarium mean length	Sp.gland condition and diameters in u	Oviposition	C.cardiaca/ allata condition
Brain N.S.C.	20	10	80 x 90	-	Atrophy	560 u	Greatly shrunk 200 x 75	NIL	Atrophy
Operated Control	10	7	210 x 100	12 x 11	Corpora lutea and resorption bodies	900 u	Full 600 x 150	Oviposited	Normal
S.O.G N.S.C.	20	9	180 x 90	11 x 13	Resorption	600	Shrunk 450 x 120	NIL	Shrunken
Control	10	5	210 x 120	11 x 12	Corpora lutea and resorption bodies	910	Full 620 x 160	Oviposited	Normal
S.O.G. N.S.C. followed by Farnesol treatment	10	5	215 x 100	12 x 12	Resorption (42%)	990	Shrunk 480 x 120	NIL	Shrunken
Control (S.O.G. N.S.C.)	5	3	195 x 75	12 x 12	Resorption (95%)	590	460 x 120	NIL	Shrunken

TABLE 10. Morphometric measurements of the reproductive organs and their condition in operated males of *T. granarium* based on all survivals.

Part of N.S. cauterized	No. of adults	No. of survivals	Length of testis in u	Testes condition	Accessory gland condition and width in u	Vesicula seminoles condition and length in u	C. cardiaca/ allata condition
Brain N.S.C.	10	6	160	Atrophy	collapsed 80	Shrunk, empty 75	Atrophy
Control	10	8	800	All stages of meioses	Distended, full, 220	Distended, full 190	Normal
S.O.G. N.S.C.	10	4	650	" At slow rate	Shrunk, empty 180	Shrunk, empty 140	Shrunk
Control	10	6	810	All stages of meioses	Distended, full, 240	Distended, full 195	Normal
S.O.G. N.S.C. followed by farnesol treatment	10	4	645	All stages of meioses At slow rate	Small, full$^{+}_{-}$ 200	Empty 200	Small
Control S.O.G. N.S.C.	5	2	640	"	Shrunk, empty 180	Shrunk, emoty 145	Shrunk

the corpora allata. Thus the atrophy of the ovarioles in T. granarium could be also due to a failure of carbohydrate synthesis. This speculation arises from the fact that carbohydrate consumption during the early stages of egg growth was low but increased during yolk formation in Calliphora (Strangeways - Dixon, 1960, 1961).

In ovipositing females, not only are brain neurosecretory cells necessary for maturation and ovarial development, but also for the continuation of ovulation and oviposition. Although these females had commenced oviposition, they stopped altogether when the brain neurosecretory cells were removed. In those females which laid eggs during the few hours after the operation, the influence of the neurosecretory cell hormone already present in the body and in the corpora cardiaca seems to have persisted for this period. Thus it seems that the secretion from the brain neurosecretory cells has a 'short term' effect and hence has to be continuously released if the processes relating to maturation, development of ovaries and oviposition are to continue.

The shrinkage and activity of the spermathecal glands, and the period of survival of spermatozoa in the spermatheca seem to be influenced by neurosecretion from the brain.

This central control by the neurosecretory cells of the brain exists in male T. granarium. Spermatogenesis, the release of spermatozoa to the vesicula seminalis and the activity of the accessory gland were all inhibited in the absence of the brain hormones. The atrophy of the testes in the absence of the neurosecretory cells of the brain may be due to a failure of protein and carbohydrate synthesis.

The depletion of the neurosecretory cell in the suboesophageal ganglion of a cauterised-brain adult suggests that the brain neurosecretory cells stimulate the activity of the ganglion to produce its hormone.

The complete disappearance of brain secretory products from the corpora cardiaca a few hours after cauterising the brain indicates that the corpora cardiaca function as a 'short period' storage organ for the brain, and the brain corpora cardiaca are functioning as a unit. The shrinkage of corpora cardiaca and allata in the absence of the brain neurosecretory cells also indicates that the full function of the glands is influenced by the secretion. In mated females, it is also

shown that even the continuation of the function of corpora cardiaca/allata appears to depend on a continuous supply of neurosecretion from the brain.

Finally, it must be mentioned that cauterisation of the brain neurosecretory cells does not apparently interfere with other physiological processes, e.g., feeding, digestion and excretion. Thus it is evident that the cessation of ovulation, spermatogenesis, atrophy of ovaries and testicular follicles and disability of copulation are largely due to the cessation of brain neurosecretion.

From the results obtained after cauterisation of the neurosecretory cell in the suboesophageal ganglion, it is evident that the ganglion does not interfere with sexual behaviour. Females and males deprived of their suboesophageal ganglion secretory cell can still be stimulated by the presence of the opposite sex and can still copulate. Also, its hormone does not initiate oocyte and spermatogonial differentiation, yet it seems to be necessary for the brain/corpora cardiaca/allata to function at a level suitable for oviposition and maturity.

The farnesol experiments suggest that the suboesophageal ganglion hormone is necessary for oviposition as the oocytes were developing, yolk was deposited and yet ovipositing females ceased to oviposit. Also, its hormone seems to be necessary for the activity of the spermathecal gland. Farnesol experiments also indicate that the corpora allata are responsible for yolk deposition and a lack of their hormone plays an important part in oocyte resorption.

In the male the suboesophageal ganglion hormone seems to influence the release of the spermatozoa to the vesicula seminalis. Cauterisation of the ganglion inhibits the activity of the corpora allata, so that it shrinks and its secretion ceases. Consequently, the accessory glands do not become filled with secretion. When farnesol was applied, the secretion in the accessory gland was restored, yet no release of spermatozoa to the vesiculae seminales occurred.

Finally it must be mentioned that the previous histological and experimental studies have thrown some light on the nature of the problem of the inter-relationship of the various neuro-endocrine centres. There is an important relation between the neurosecretory system and reproduction in adult T. granarium. Cessation of ovarial

development, ovulation and oviposition in the female and of maturation and spermatogenesis in the male are all effects which follow disturbances of the neurosecretory system.

XI THE ROLE OF THE MALE IN INDUCING OVIPOSITION

Introduction

Virgin females of T. granarium do not lay eggs. Copulation is followed by a short pre-oviposition period. The majority of the eggs are deposited during the first few days of the oviposition period. The daily egg number then decreases rapidly and the females live a few more days in the post oviposition period, without laying eggs, before death (see Table 11. below).

Table 11. Number of eggs and oviposition period of females after a single copulation.

Female number	Daily egg number								Number of eggs per female	Oviposition period in days
	1	2	3	4	5	6	7	8		
1	16	6	2	0	1	0	0	+	25	4
2	6	14	5	3	2	0	0	+	30	5
3	10	14	2	2	1	0	+		29	5
4	16	8	2	1	0	0	+		27	4
5	10	14	3	2	0	0	0	+	29	4
6	9	16	5	0	0	0	0	+	30	3
7	16	10	3	3	1	0	0	+	33	5
8	15	9	5	2	0	0	0	+	31	4
9	13	13	3	1	0	+			30	4
10	13	10	4	2	0	0	0	+	29	4

Mean oviposition period in days = 4·2

Mean number of eggs per female = 29·3

+ = death of female.

Hadaway (1945) maintained that continued copulation throughout the oviposition period is unnecessary for the attainment of full potential fertility. He added that a single copulation is sufficient to fertilise all the eggs and females isolated from males after starting oviposition lay as many eggs as females left with males throughout life. Hadaway

indicated that the rate of oviposition and fertility of the egg laid, also remained unchanged. The data recorded in the preceeding table, however, pose certain problems. Why does the daily egg number decrease so rapidly after the second ovipositing day ? Is it because of the lack of mature oocytes; or does it depend on the quantity and 'period of survival' of the spermatozoa in the spermatheca ?

Dissection of these ovipositing females revealed the presence of crowded living spermatozoa in their spermatheca in the first two ovipositing days. Corpora lutea were also found at the basal of all the ovarioles. In the third and fourth days, however, the living spermatozoa decrease in number conspicuously and quite a few mature oocytes became resorbed instead of being ovulated and fertilised. Experiments were carried out to attempt to determine whether the oviposition period could be prolonged and the number of eggs increased if these females were provided with another supply of fresh spermatozoa; the effect of varying the interval between successive copulations was also investigated.

Experiment I

Re-introduction of males after one day of oviposition.

Materials and technique

Sixteen mature males were introduced to sixteen one-day old ovipositing females and were isolated from the males immediately after the first copulation. Their daily egg number was recorded. Each couple was put in a 2" by 1" tube and provided with black paper folded concertina-fashion to serve as an oviposition site. All were raised at 30° C. and 75% R.H. The insects were observed in copulation. The sixteen couples copulated within the first hour after they were put together and then the males were removed. Daily inspections were made of the oviposition sites and the length of the oviposition periods were recorded. Six ovipositing females were fixed on the third and fourth oviposition days for histological studies.

Results

The number of eggs and the oviposition periods are tabulated below.

Table 12. Oviposition periods and number of eggs of females re-introduced to males after the first ovipositing days.

Female number	Daily egg number								Number of eggs per female	Oviposition period in days
	1	2	3	4	5	6	7	8		
* 1	14	13	4	2					(33)	(4)
2	16	10	5	4	1	0	+		36	5
* 3	5	14	6						(25)	(3)
4	9	13	10	2	0	1	+		40	5
5	12	14	3	0	1	0	0	+	30	4
* 6	13	10	4						(27)	(3)
7	16	16	3	2	0	1	0	+	38	5
8	14	12	6	1	1	0	+		34	5
* 9	6	14	8	0					(28)	(3)
10	13	14	4	3	0	0	+		34	4
11	12	12	6	2	0	+			32	4
* 12	10	8	8	1					(27)	(4)
13	12	0	17	4	4	0	0	+	37	4
14	5	15	8	2	0	1	0	+	31	5
* 15	8	12	2	2					(24)	(4)
16	5	10	4	4	1	0	+		24	5

Mean oviposition period in days = 4·6

Mean number of eggs per female = 33·6

\+ = death

* = fixed

Dissection revealed the presence of quite a few living spermatozoa in the spermatheca on the third oviposition day. On the fourth day, however, the number of the living spermatozoa decreased

rapidly and a fairly large number of them were nonmotile. Very few resorption bodies were found on the third day but they increased on the fourth day.

Experiment II

Re-introducing the males after two oviposition days.

This experiment was undertaken in the same way as the previous one and the results are tabulated below.

Table 13. Oviposition periods and number of eggs of females re-introduced to males after two oviposition days.

Female number	Daily egg number								Number of eggs per female	Oviposition period in days
	1	2	3	4	5	6	7	8		
* 1	13	8	8	6					(35)	(4)
2	5	16	6	10	4	0	+		41	5
3	16	9	8	8	7	2	0	+	50	6
4	13	9	9						(31)	(3)
* 5	10	6	16	8	3	1	+		44	6
6	16	6	13	9	1	3	+		48	6
7	15	3	10	10	5	1	0	+	44	6
* 8	16	9	9	6					(40)	(4)
9	15	7	10	2	2	0	0	+	36	5
*10	16	0	10						(26)	(3)
11	4	14	12	8	1	0	+		39	5
12	13	5	13	6	3	0	+		40	5
*13	13	7	7	4					(31)	(4)
14	8	14	9	7	2	0	+		40	5
15	5	17	17	6	4	1	+		50	6
* 16	16	5	9						(30)	(3)

Mean oviposition period in days = 5·5

Mean number of eggs per female = 43·2

\+ = death

* = fixed

The figures between brackets are omitted from the calculations of the mean values.

Dissection revealed the presence of crowded living spermatozoa in the spermathecae on both third and fourth oviposition days. No resorption bodies were ever found in the specimens examined.

Experiment III

Re-introducing the males after three oviposition days.

Here again sixteen males were introduced to sixteen three-day old ovipositing females. They were treated as in the previous experiments. The results are tabulated below.

Table 14. Oviposition periods and number of eggs of females re-introduced to males after the third ovipositing day.

Female number	Daily egg number								Number of eggs per female	Oviposition period in days
	1	2	3	4	5	6	7	8		
1	14	6	3	5	4	0	0	+	32	5
* 2	5	16	2	6					(29)	(4)
3	6	16	3	8	1	0	+		34	5
* 4	13	7	2						(22)	(3)
* 5	8	8	1	8					(25)	(4)
6	2	11	5	10	1	1	0	+	30	6
7	11	1	14	8	1	+			35	5
8	16	16	3	8	2	0	+		45	5
* 9	8	14	2	9					(33)	(4)
10	16	5	5	11	1	0	+		38	5
11	15	10	7	7	2	0	+		41	5
12	16	2	2	11	6	1	+		38	6
*13	7	15	2						(24)	(3)
*14	14	2	5						(21)	(3)
15	5	5	17	2	3	1	0	+	33	6
16	15	16	0	4	6	1			42	5

Mean oviposition period in days = 5·3 + = death

Mean number of eggs per female = 36·8 * = fixed

The figures between the brackets are omitted from the calculations of the mean value.

Dissection showed the presence of a few living spermatozoa and a large number of resorption bodies on the third oviposition day. On the fourth oviposition day, however, the number of living spermatozoa increased and from 1 - 3 resorption bodies were found in each specimen examined.

Experiment IV

Males kept with females throughout the whole period of life.

In this experiment ten males were mated with ten females. Each couple was kept in 2" by 1" tube provided with a suitable oviposition site. All were raised at 30° C. and 75% R.H. Daily inspections were made of the oviposition sites and the length of the oviposition periods was recorded.

Table 15. Oviposition periods and number of eggs of females kept with males throughout their whole period of life.

Female number	Daily egg number								Number of eggs per female	Oviposition periods in days
	1	2	3	4	5	6	7	8		
1	17	6	3	8	2	0	+		35	5
* 2	16	8	5	7					(36)	(4)
3	13	12	10	3	2	0	0	+	40	5
4	9	16	12	6	1	1	0	+	45	6
* 5	14	7	10	2					(33)	(4)
6	16	11	8	8	3	2	0	+	48	6
* 7	15	7	10	3					(35)	(4)
8	8	14	4	7	1	0	+		34	5
9	18	16	6	4					44	(4)
10	16	11	8	6	2	1	0	+	44	6

Mean oviposition period in days = 5·3

Mean number of eggs per female = 40·2

\+ = death of females.

* = fixed

Dissections revealed various numbers of corpora lutea and

resorption bodies in all the females examined. In this experiment, however, it is not certain whether all the females copulated more than once.

Discussion

The egg count and the oviposition data were analysed using the 't test' and differences between observations were found to be highly significant. This indicates that more than one copulation is necessary to obtain the maximum number of eggs and the longest period of oviposition. This seems to contradict the statements of Hadaway (1945). It is also found that the second copulation should take place after the second oviposition day to ensure maximum output of eggs and the longest oviposition period.

Copulation in T. granarium depends on maturity and age. Newly emerged females copulate immediately after males are introduced to them. As they grow older, they take a longer time before they copulate.

The above experiments suggest that the 'period of survival' of spermatozoa and the rate of egg development played a great part in increasing the number of eggs laid by a female. The 'period of survival' of the spermatozoa of T. granarium in the spermatheca is approximately two days. After this time spermatozoa are found in the spermatheca but many of them are immobile. Thus the length of oviposition period and number of eggs laid by a female T. granarium does not depend on the quantity of spermatozoa in the spermatheca.

XII EFFECT OF SUBLETHAL DOSES OF PYRETHRUM AND D.D.T. ON THE NEUROSECRETORY SYSTEM

INTRODUCTION

Pyrethrins and DDT have long been known as nerve poisons. The effects of both insecticides on the ganglia have been confirmed by a number of workers; e.g. Krüger (1931) in Carethra, Hartzell (1934, 1935) in mealworms, Melanoplus, Tenebrio and other insects, Wigglesworth (1941) in Rhodnius and Chang (1951) in Periplaneta and Apis mellifera. They all recorded different grades of lesions or vacuolation and degeneration in different ganglia of the central nervous system in these insects after treatment with lethal doses of pyrethrum or DDT. Richards and Cutcump (1945) working with pyrethrins obtained similar results in paralyzed Periplaneta but concluded that it was questionable as to whether the pyrethrins poisoning had any casual relationship to degeneration other than killing the nerves. They also were unable to detect any clear cut evidence of degeneration in the nerve cord of moribund Periplaneta after treatment with DDT. Similarly moribund Calliphora and Anopheles did not show any evidence of histological changes from DDT poisoning,(Witt, 1947; Jones, 1953)

Welsh and Gordon (1947), working with crustaceaus and Periplaneta, showed that Pyrethrins and DDT acted upon the sensory nervous system and their primary action was to cause an increase of afferent discharges, leading to hyperactivity and eventual reflex incoordination.

Blum & Kearns (1956) found that blood from Periplaneta americana prostrated by pyrethrum was found to be toxic when injected into sarcophaga crassipalpis but no pyrethrum was found in the blood. They maintained that the toxicity of the blood was due neither to the pyrethrum nor to a metabolite of pyrethrum.

In 1958 Colhoun reported that blood from cockroaches prostrated by DDT contain five active substances; two of them are a hormone from corpora cardiaca and another from corpora allata. In 1959 Colhoun reported that treating Periplaneta with DDT caused depletion of a hormone that accumulated in the blood. He suggested that this hormone might be responsible for part of the toxicity of such blood. He also

stated that only corpora cardiaca extracts excited isolated nerve cords as did blood from DDT-poisoned cockroach.

In 1959 Sternburg et al. reported the presence of a neuroactive substance which was released into the blood of Periplaneta Americana during the course of DDT poisoning. They maintained that the source of the toxicant is apparently the central nervous system itself during periods of great nervous activity due to excessive afferent impulses generated in the sensory nerves by direct action of DDT. These authors confirmed their results by electrical stimulation of isolated thoracic and abdominal nerve cords which also resulted in the release of the neuroactive substance that has the same biological, chromatographic and chemical properties as the DDT-induced substance.

In 1947, Tenhet reported that the cigarette beetle, Lasioderma serricorne, surviving exposure to a pyrethrum-oil spray consistently deposited fewer eggs than untreated beetles.

Kucukkoca (1952) reported that some females of Trogoderma versicolor obtained from larvae treated with sublethal doses of pyrethrum and DDT laid fewer eggs than those developed from untreated larvae. Whereas some females of Dysdercus fasciatus obtained from the nymphs which had been treated with the same doses of these two insecticides, laid more eggs than the untreated ones. Some individuals of Trogoderma treated in the larval stage, he added, developed to deformed adults.

In 1954 Shantaram working also on Trogoderma versicolor confirmed the previous results and added that low doses of these insecti-cides stimulated the egg-laying. He also maintained that the insecticidal treatments retarded the development of the larvae and these consumed less food than the untreated ones. No difference was noticed in the frequency of moulting and no appreciable effect was observed either on the pupal duration or on the length of the adult life after treatments.

Risella oil, the carrier for the pyrethrum and DDT, when applied for control experiments gave similar results (Kucukkoca 1952, & Shantaram 1954).

There is at least a superficial similarity between the effects of poisoning with sublethal doses of pyrethrum and DDT. and the endocrinal syndrome recorded in the present work. Thus an examination

of this similarity to see how far the two syndromes coincide seemed likely to be fruitful.

MATERIALS AND TECHNIQUE

Mature larvae were used. Pyrethrins applied in this work were obtained from Messrs. Stafford Allen and Sons Ltd. as an extract containing approximately 20% weight/weight pyrthrins. Pure DDT (M.P.107-8°C) was obtained by recrystallizing commercial samples. Four per cent stock solutions of pyrethrum and DDT were separately prepared by dissolving the insecticides in Shell Risella oil 17 and then diluted with four times their volume of "Analor" petroleum ether to provide an even distribution over 7 cm Whatman No. 1 filter papers (R.E. Blackith, 1950). Risella oil is a heavy paraffinic oil substantially free from aromatic and unsaturated hydrocarbons and having a very high unsulphonated residue. Each insecticide was applied in three different dosages; 2 per cent, 1.0 per cent and 0.5 per cent. The 4 per cent stock solutions of pyrethrum and DDT were diluted to the required concentration by a solvent containing 20% Risella oil and 80% petroleum ether. Assay was made by the crawling insect method of Blackith (1950). The filter paper was placed on a glass plate and 0.5 ml. of insecticide solution was spread evenly in a spiral by drawing the tip of the pipette over the paper. Within 2-4 minutes of application, the bulk of the petroleum ether evaporated, leaving the filter paper impregnated with a solution of the insecticide in Risella oil. The papers were left for a further period of at least 30-40 minutes to allow the last traces of the petroleum ether to evaporate.

Mature larvae in each experiment, all drawn from the same generation, were dropped on the treated filter papers. The insects were confined on the papers by brass rings 2.5 cm. deep and 6.4 cm. internal diameter, having the internal surface nickel-plated and polished. The insects were raised at 33°C and 70% RH and were maintained in contact with the insecticide for 24 hours. At the end of the exposure period insects were classified as 'paralysed' and 'not paralysed'. The brass rings were used to sweep the larvae into a pile in the middle of each paper and a 15 watt bulb lamp was placed 8 cm. above the larvae for two minutes; larvae unable to leave the centre of the paper were counted as 'paralysed', while those that crawled to the edge of the paper were counted as 'not paralysed'.

From each dosage, a few paralysed larvae were fixed for further examination while the rest were transferred to clean food in separate jars, where they were allowed to remain at 33°C and 70% RH. The conditions already known to extend the larval duration in T. granarium such as low temperatures, lack of fresh food and starvation were avoided in these experiments. The insects were examined daily and newly emerged adults of each sex were introduced in pairs in plastic petri dishes. Eggs laid were counted daily; adults not copulating were fixed for histological studies.

Control experiments:

Control experiments were performed in the same way. The insecticides, however, were omitted and only the mixed solvents 1 : 4 of "Shell Risella 17" oil and petroleum were employed.

RESULTS

All dosages both of Pyrethrum and of DDT resulted in similar morphological, physiological and histological changes in treated larvae. The degree of these changes, however, varied directly with the concentration of the insecticides used, and varied slightly when pyrethrum was used than DDT, which may be due to the chemical instability of the pyrethrins molecules. Larval duration of 20% of the treated insects was prolonged to 2-3 weeks. Such larvae decreased in size and some of them moulted 2-4 times before pupation. Pupal duration was also lengthened; some pupae either being deformed or producing deformed adults (Figs. 85 & 86).

The number of deformed adults was higher and the percentage killed was lower after treating the larvae with 1.0% and 0.5% of insecticides, whereas 2.0% resulted in more deformed pupae and higher percentage killed.

Deformations of the pupae were exhibited mainly by the cuticle which became covered with setae and bristles resembling the larval setae. Deformaties in the adult were exhibited by the wings, genitalia and the cuticle. The elytra were displaced and weakly sclerotized. The genitalia were extruded and hook shaped. The cuticle was less sclerotized than in normal adults and was covered with setae resembling the larval setae.

Deformed pupae never became adults and deformed adults never copulated even if normal adults of different sexes were introduced to them. Deformed adults behaved exactly as those who had their brain neurosecretory cells cauterized. Whenever a normal male mounted a deformed female, he was always rejected and when a normal female approached a deformed male she was always refused.

Surviving undeformed adults copulated and females laid eggs. However, the number of eggs produced was appreciably smaller than normal. The following table shows the number of eggs laid after the different insecticidal treatments.

Treatment	No. of Female	Total No. of eggs laid	Treatment	No. of Female	Total No. of eggs laid
Oil	20	525	untreated larvae	20	724
0.5% Pyrethrum	20	475	0.5% DDT	20	445
1.0% "	20	410	1.0% "	20	375
2.0% "	20	355	2.0% "	20	301

Histologically, paralysed insects show tremendous nerve lesions. The brain, suboesophageal ganglion, corpora cardiaca/allata and their connectives are highly vacuolated and disintegrated (Fig. 87). These symptoms are seen more clearly in the medullary zone than the cortex. The neurosecretory cells decreased in size and not more than three cells in each hemisphere of the brain could be identified. The neurosecretory products in these cells were rather depleted (Fig. 88).

Larvae which were not paralysed by the insecticide treatments produced pupae and adults, having normal appearance. In such individuals, however, it appears that the different dosages of insecticides did not prevent the neuro-endocrine system from functioning but caused a slight inhibition in the activity of the system (see table 16). The neurosecretory cells in the larva, pupa and the adult became inactive synthetically and in a state of low release. The cells decreased in size and contained few aggregates of secretion and the amount of their products carried along their axons was rather small (Figs. 89, 90 & 91).

The corpora cardiaca in the three stages decreased in size and all contained very little brain neurosecretion. The corpora allata also showed a slight decrease in size and the cells were packed closely indicating inactivity of the gland.

Dissection of the abdomens revelaed the presence of very small amounts of food in the gut of the larvae. In the adult, dissection showed different stages of yolk resorption and shrinkage in the ovaries in the female and a slight decrease in the testes of the male.

No spectacular changes have been observed in control insects.

DISCUSSION

The histological effects of pyrethrum and DDT on the endocrine system in paralysed larva, described in the present work, are similar to the findings of previous workers investigating the effects of lethal doses of insecticides on different insects; e.g. in Melanoplus and Tenebrio (Hartzell, 1934, 1935), Rhodnius (Wigglesworth, 1941) and Periplaneta and Apis mellifera (Chang 1951). One, however, should acknowledge that some of these effects in moribund insects may be secondary effects produced in dying tissues.

The morphological and biological results obtained after examining the surviving larvae and the growing adults are more or less in agreement with the previous findings obtained by Tenhet (1947) in the cigarette beetle and by Kucukkoca (1952) and Shantaram (1954) in T. versicolor. However, in the present work, sublethal doses of pyrethrum and DDT have been shown to cause the larva to moult about three times before pupations and to cause prolongation in the pupal duration and always resulted in depositing fewer eggs than normal. Whereas Shantaram (1954) maintained that no difference was noticed in the frequency of moulting and no appreciable change in the pupal duration was observed after insecticidal treatments. He also recorded that low doses of both insecticides stimulated the egg-laying.

The results obtained in the present work indicate that there is a correlation between different physiological processes, including feeding, growth, metamorphasis and reproduction and the activity of the neuro-endocrine system in T. granarium. The release of hormones under ordinary conditions results in a proper integration of physiological events; but when released in greater or lesser amounts than normal, these hormones may lead to either paralysis or abnormal behavioral characteristic.

In paralysed larvae the neurosecretory cells and corpora cardica and allata have been shown suddenly depleted within 24 hours from the time of treatment. This sudden excessive release of secretory products as a result of intensive stress may be the direct cause of the paralysis. The toxic effect of different neuroactive substances secreted by various parts of the central nervous system has been shown in other insects by numerous workers; e.g. in Periplaneta americana by Cameron (1953), Harker (1954), Colhoun (1958, 1959) and Sternburg et al. (1959 & 1963).

In the larvae which were not paralysed, it seems that the insecticides effect is not as strong as in the above case and apparently the synthetic activity of the neurosecretory is hindered and thereby the rate of release is reduced. Thus physiological processes are disturbed.

Ozbas and Hodgson (1958) reported that the brain and the corpora cardiaca produce a neuroactive substance. When applied to isolated cockroach nerve cord the level of spontaneous acticity decreased; when injected in cockroach this assumed abnormal postures.

Less food was consumed by insects in which the activity of the endocrine system was hindered. In Calliphora the neurosecretory cells of the brain and the corpora allata have been shown to be necessary for the ingestion of protein and carbohydrate respectively (Strangways-Dixon 1961b).

Insecticidal treatments results suggest that pyrethrum and DDT upset the normal activity of the endocrine system in favour of the corpora allata, and more juvenile hormone is secreted. This is in agreement with the observation that some of the larva moulted 2-4 times, retaining their larval characters for about four weeks. The effect of juvenile hormone on metamorphosis has been discussed in previous chapters and has been demonstrated by many previous workers (see Wigglesworth 1934, 1936, 1954b and 1964).

The disturbance of the hormone balance in favour of juvenile hormone also resulted in the occurence of deformed pupae and adults (Metathetely). Similar abnormalities have been demonstrated and discussed in the present work; see Chapter VIII.

DDT and pyrethrum seem to hinder the activity of the neurosecretory in adult T. granarium. Thus egg development and spermatogenesis were retarded.

The foregoing pages indicate that hormonal release normally must be precise in quantity and time; abnormal release may cause profound pathological and physiological consequences.

TABLE (16) MEASUREMENTS OF THE NEURO-ENDOCRINE AND REPRODUCTIVE ORGANS IN T. GRANARIUM; BASED ON EXAMINATION OF 4-5 INDIVIDUALS OF EACH STAGE.

	Treatments		
	Insecticides	Oil (Control)	Normal
		LARVA	
Diameters of neuro-secretory A-cells	7.0U X 8.5 U	9 U X 11 U	11 U x 12 U
Vol. of corpus cardiacum	78,990 U^3	87,860 U^3	89,320 U^3
Vol. of corpus allatum	30,000 U^3	37,000 U^3	38,130 U^3
		PUPA	
Diameters of neuro-secretory A-cells	8.5 U X 9 U	10 U X 11 U	11 U X 13 U
Vol. of corpus cardiacum	29,760 U^3	34,800 U^3	34,950 U^3
Vol. of corpus allatum	21,680 U^3	28,710 U^3	28,714 U^3
		ADULT	
Diameters of neuro-secretory A-cells	8.5 U X 9.5 U	11 U X 12 U	13 U X 15 U
Vol. of corpus cardiacum	86,000 U^3	96,900 U^3	98,760 U^3
Vol. corpus allatum	99,200 U^3	145,800 U^3	148,420 U^3
Length of vitellarium	1,500 U	1,750 U	1,800 U
Length of testis	700 U	900 U	900 U

XIII GENERAL DISCUSSION

Some aspects of the present investigations have already been discussed in their respective chapters. This general discussion is, therefore, concerned with the histological structure of the neurosecretory system, the neurosecretion - growth - reproduction relationship in the light of other known work and environmental effects on the endocrine activity.

The nervous system in T. granarium evidently grows by a combination of increase in cell size and in cell number. The ganglion cells show a progressive but very slight increase in size. This is in agreement with the present knowledge of other insects, e.g., Bombyx mori (Trager, 1935a), Popillia japonica (Abercrombie, 1936) and Aëdes aegypti (Trager, 1937), and differs from the findings of Murry and Tiegs (1935) in Calandra oryzae, of Trager (1935a) in Lucilia sericata and of other investigators in different insects where growth of the nervous system is attributed solely to multiplication.

The principal anatomical features of the neurosecretory system of T. granarium are comparable to those in other insects described by Cazal (1948). The most prominent groups of neurosecretory cells are the medial cells situated in the pars intercerebral region of the brain. They consist, as in most insects, of two groups which can frequently be mistaken for a single group. Their existence in two groups is confirmed by the decussation of their axonal pathways. Two types of cells have been found, by the accepted staining procedures, persistently distinct from one another. These two types are A and B cells and they are found in all the stages except the first, second and third-instar larvae where A cells only are found. The number of A and B cells varies from one instar to another and from one stage to another. However, no variation in their numbers throughout one stage's life, which would indicate transformation of one type to another, could be found. In other insects, investigators have recorded the existence of further types of cells named C and D cells in the pars intercerebral region of the brain, e.g., O. fasciatus (Johannson, 1958), S. gregaria (Highnam, 1961) and Dermestes (Ladduwahetti, 1962).

The lateral groups of neurosecretory cells in T. granarium

are comparable to those demonstrated in Hyalophora cecropia (Williams, 1948), Calliphora erythrocephala (Thomsen, 1952), Iphita limbata (Nayar, 1955), Adelphocoris lineolatus (Ewen, 1962), Dermestes (Ladduwahetti, 1962) and G. tanaceti (Siew, 1963). However, the distribution of cell types found in this group of neurosecretory cells seems to vary in the few insects which have been studied. In Iphita, the lateral cells are B - cell type, in A. lineolatus, there are only three B cells, in Dermestes, one group contains four B cells and one C cell and in G. tanaceti there are 2 - 3 A cells and a highly variable number of D cells. In T. granarium, the lateral group of cells appears for the first time in the third instar larva and they are found, also, in the successive instars and stages. In the first and second-instar larvae, however, there is no trace of these cells. It cannot be ruled out that the apparent absence of the lateral group cells in these two instars may be due to the limitation of the techniques used. The number of the neurosecretory cells in the lateral group differs from one stage to the other, but in all, the cells are of the A - cell type.

The A and B cells of T. granarium fall into the general category of A and B cells described in all insects studied so far. Those cells that stain blue-black with chrome haematoxylin/phloxine and purple with paraldehyde fuchsin are designated as A cells. The B cells are phloxinophil and stain red with chrome haematoxylin/phloxine and bluish green with paraldehyde fuchsin. There is still, however, some controversy as to whether the different types of secretory neurones are in reality distinct cell types or merely phases in the secretory cycle of a single type. Thomsen (1954) considered the phloxinophil stage to be a phase in the secretory cycle of the Chrome haematoxylin positive cells. Her views were supported by the detection of both Chrome haematoxylin positive and phloxin positive material within the same cell (Thomsen, 1954; Lhoste, 1952); also by the transformation of materials from Chrome haematoxylin positive to phloxin positive, during the course of migration along the axons of certain Crustacea (Carlisle, 1953) and by the observation that in castrated females of Leucophaea, Aldehyde fuchsin positive cells changed to Light-green positive (Scharrer, 1955). This view has been further favoured by de Lerma (1956), Herlant-Meewis and Paquet (1956) and Brandenburg (1956). Kopf (1957) considers that in

Drosophila this is true of the cells of the pars intercerebralis but not of the phloxinophil cells of the lateral groups and those of other ganglia. Nayar (1955) has suggested that in Iphita the B cells are A cells deprived of the bulk of their secretion. In T. granarium, the two types of cells have been found to be persistently distinct from one another throughout the different stages of the life cycle.

Apart from the two groups, median and lateral, a few workers reported the presence of other groups of neurosecretory cells in the brain. Dupont-Raabe (1956) detected one or two neurosecretory cells in the tritocerebrum of Carausius morosus; Fraser (1959) described six groups in the brain of Luciliar caesar; Khan and Fraser (1961) found ventral cells in the brain of Periplaneta americana; and Ladduwahetty (1962) described another group located in a ventro-posterior position in the brain of Dermestes and termed it the ventral group.

The axons from the two medial groups of neurosecretory cells in T. granarium give rise to the nervi corporis cardiaci I which is termed by other workers the cerebro-cardiac internus nerve, NCCI. Each group gives rise to the nerve of the opposite side and delivers their secretion in the corpus cardiacum. Thus each gland is innervated by axons from the opposing medial group of neurosecretory cells. This condition seems typical in all insects studied so far (Van der Kloot, 1960). The axons from the lateral groups of neurosecretory cells, however, run directly and give rise to the cerebro-cardiac externus nerve, NCCII. This is in agreement with most of the insects previously studied. In a few insects, however, the origins of the cerebro-cardiac externus nerves have been traced to some medial neurosecretory cells; e.g., Cicadas and Coccids (Cazal, 1948), the larva of Tabanus (Thomsen, 1951) and in Andrena vaga (Brnandenburg, 1956). Ladduwahetty (1962) maintained that in Dermestes one neurosecretory cell from the lateral group diverts to join the pathways from the medial group to the NCCI.

A third cerebral pathway has been detected in Leucophaea maderae (Engelmann and Luscher, 1957) and in Hayalophora cecropia (Stumm-Zollinger, 1957). Although, these two cases these pathways have been seen to join the pathways to the NCCI, and no crossing of pathways from one hemisphere of the brain to the other has been observed, their cells of origin have not been traced. In Carausius morosus,

Dupont-Raabe (1956) also detected a third cerebro cardiac nerve which derives from a few neurosecretory cells in the tritocerebrum. Also, in Oncopeltus, (Johannson, 1958) and in G. tanaceti (Siew, 1963), a third cerebro cardiac nerve has been observed to leave the posterior ventral part of the brain, but its origin has not been traced. A third cerebro cardiac nerve which arises from the region of the tritocerebrum has been noted by Willey (1961) in Periplaneta.

The suboesophageal ganglion attracted little attention of neurosecretion investigators compared with the attention paid to neurosecretion in the brain. The only reports of the occurrence of neurosecretory cells in the suboesophageal ganglion are by Scharrer (1941), Harker (1955, 1960), Fuller (1960) and Delphin (1963) in Orthoptera; by Rehm (1955) in Lepidoptera; by Nayar (1955), Johannson (1958) in Hemiptera and by Kirchner (1960), Siew (1963) in Coleoptera. The number and type varies in these insects. A - cells are found in all of them except Oncopeltus fasciatus (Johannson, 1958) where B - cells only are found. B - cells are also found in three other insects located in the ganglion among the A - cells. In T. granarium there is only one A - cell located in the ventral mid-line in the suboesophageal ganglion of the first - fourth instar larvae, pupa and adults. In mature larvae, however, six neurosecretory C - cells appear surrounding the A - cells. It is of interest that in Dermestes, from the same family as Trogoderma, Ladduwahetty (1962)reported the absence of any neurosecretory cells in the suboesophageal ganglion.

The neurosecretory pathways of the suboesophageal ganglion in T. granarium have not been traced successfully. There is, however, evidence that the axons of the cell run deeply into the neuropile, beyond which their direction is unknown. This has been stated in most insects studied so far. Delphin (1963) however, showed experimentally that the axons of the neurosecretory cells in the suboesophageal ganglion of S. gregaria traverse backwards along the ventral nerve cord.

The corpus cardiacum in T. granarium larva, pupa and adult does not show any differentiation into an anterior and a posterior region as has been detected in most insects (Cazal, 1948). However, two zones could be identified in the gland. In inactive larva and adult the two zones are ill defined whereas in the pupa they are always clearly

definable zones. The two zones are the cortex and the medullary. Two types of cells are demonstrated in the corpus cardiacum. The larger secretory cells which no doubt correspond to the 'chromophile cells' described by Cazal (1948) and the smaller non secretory cells which are comparable to the 'chromophobe cells' of Cazal (1948). These two types of cells have been detected by most workers in different insects. A third cell type, however, has been described by Johannson (1958) in Oncopeltus as 'chromophobe glia like cells'.

The axons from NCCI and NCCII intermingle in the anterior portion of the corpus cardiacum. Some of these axons traverse their entire length into the corpus allatum. These axons may be considered to constitute the cardiaco-allatum nerve which is very distinct in some insects as in Hydrous piceus (Cazal, 1948). However, in T. granarium a corpora cardiaca/allata nerve has never been found. In Dermestes this nerve is clearly shown by Ladduwahetty (1962).

Neurosecretory material from the A and B cells of the brain can be seen in the corpus cardiacum of T. granarium. This is similar in all insects previously studied.

The corpus allatum in T. granarium consists of compact cells invested by a thin membrane. One type of cell is found in the gland. This is in agreement with the findings of other investigators in many insects. On the other hand Mendes (1948) describes four types of cell in the corpus allatum of Melanoplus differentialis. In Trogoderma, the cell outlines are discernible but their visibility depends on the activity of the gland. Cell outlines have been also discerned in the corpus allatum of Rhodnius (Wigglesworth, 1934), Oncopeltus (Johannson, 1958) and in Dermestes (Ladduwahetty, 1962). Conversely, Nayar (1956b) maintained that the gland in Iphita is syncytial. The orientation of the cells within the gland in Trogoderma and the presence of vacuoles also depend on the activity of the corpus allatum. In active glands the cells are larger and distributed heterogeneously with an enlargement of the intercellular spaces and vacuoles are discernible. In inactive glands, the cells are smaller, their outlines are discernible with difficulty and they are closely packed and homogeneously distributed. This is in agreement with the findings in Melanoplus (Mendes, 1948) and Dermestes (Ladduwahetty, 1962). In Rhodnius, however, the vacuoles were present

only when the glands had shrunk and were inactive (Wigglesworth, 1934).

Neurosecretion from the brain has been observed in the corpus allatum in T. granarium. This is contrary to the condition observed in T. tanaceti (Siew, 1963) but in agreement with the findings of Arvy et al. (1953) in Bombyx mori, Arvy and Gabe (1954) in Leptinotarsa, Scharrer (1961) in Leucophaea and Khan and Fraser (1962) who have shown that careful timing is necessary to demonstrate the presence of material in these organs in embryonic stages of Periplaneta americana. There is also a substance which stains strongly purple with Paraldehyde fuchsin in the corpus allatum of Trogoderma larva but is not discernible in the pupa and adult. This substance is found mostly in the peripheral parts of the gland and has rarely been found in the centre. Fraser (1959) working on the larva of Lucilia recorded the presence of a similar substance in the corpus allatum. His explanation is that this substance is associated with the tracheae which richly supply the gland in insects about to moult; the tracheal chitin is attacked by moulting fluid and the chitin precursor in the tracks of new tracheae is strongly coloured by the purple stain. This, presumably, is the case in T. granarium as the substances seem too large to be granules from the brain and they are only located peripherally.

Intercellular secretion is seen clearly in active glands. Similarly, Nayar (1954, 1956d) found prominent secretory granules in the corpus allatum of Iphita libata. Ladduwahetty (1962) proved expermentally that corpora allata of Dermestes exert an independent action, but she failed to detect any intercellular secretion. She explained the disappearance of the secretion by being soluble in either the water or the alcohol used in the technique. In Lymenoptera (Thomsen, M., 1954), Oncopeltus (Johannson, 1958) and Forficula (Lhoste, 1952), no signs of cytoplasmic secretory activity such as the appearance of granules or of vacuoles have been seen. The prothoracic glands in T. granarium are composed of cells. This is in agreement with the findings of many workers in different insects. It is of interest that in Anthrenus vorax (Dermestidae) Srivastava (1959) recorded that the prothoracic glands are syncytial.

FUNCTIONAL SIGNIFICANCE OF THE NEURO-ENDOCRINE SYSTEM

In T. granarium the neuro-endocrine system is considered to function as a whole unit for growth, metamorphosis and a full development and activity of the reproductive system. If one centre ceases to function, the remaining parts of the system are affected, thus physiological processes are disturbed. This suggests a delicate relationship and interdependence of one neurosecretory centre on the others, involving probably complex feed back mechanisms. The general effect of the endocrine system entails certain special effects which may be considered separately.

1. The brain hormones in growth, metamorphosis and reproduction.

The brain, generally, and its neurosecretory cells in particular have been found to exert an overall controlling influence on these different physiological processes.

In T. granarium larva, cauterisation of the neurosecretory cells in the brain prevents the larva from moulting and pupating. This control has been previously shown by Kopeć (1917, 1922) in Lymantria, Kühn and piepho (1936) in Ephestia, Plagge (1938) in Deilephila, Wigglesworth (1939, 1940) in Rhodnius, Schmieder (1942) in Trypoxylon, Williams (1946) in Hyalophora cecropia, Possompès (1953) in calliphora and by Nayar (1956) in Iphita. On the other hand, extirpation of the neurosecretory cells of the pars intercerebralis of Oncopeltus did not prevent larval moulting. It was assumed, however, that the neurosecretory material which, in this insect, is stored in the wall of the aorta, was sufficient for this purpose (Johansson 1957).

In dormant larva of T. granarium, cauterisation of the neurosecretory cells in the brain impedes termination of dormancy in a favourable external environment. This suggests that a certain level of hormones from the brain is essential to initiate the break of dormancy. Similarly, the brain is the centre which terminates diapause in Platysamia (Williams, 1942, 1946 and 1952), Tryllus campestis (Sellier, 1949) and in Mimas tiliae (Highnam, 1958). On the other hand some diapausing pupae could be induced to resume their development, in the absence of the brain, by implantation of active corpora allata; e.g. Hyalophora (Williams, 1959), Bombyx mori, (Kobayashi and Yamashita, 1959) and Philosamia (Nishiitsutsuji-Uwo, 1959); but it was assumed that the implanted corpora allata contained neurosecretory materials that have originated in the brain.

In adult T. granarium the neurosecretory cells in the brain exert an overall influential control on the reproductive system of both females and males. Cauterisation of these cells causes a complete atrophy and degeneration of both ovaries and testes. This is believed to be the first record of such an extreme effect of neurosecretory cells on the ovaries and testes in any neurosecretion-reproductive relationship studied. The previous workers have all come to one conclusion; namely that removal of the neurosecretory cells of the brain only inhibits the development of the ovaries, e.g. Thomsen E. (1952) in Calliphora erythrocephala, Gillett (1958) in Aedes aegypti, Ladduwahetty (1962) in Dermestes and Highnam (1962) in S. gregaria. Conversely the ovaries develop in the absence of the neurosecretory cells in Rhodnius (Wigglesworth, 1936), Carausius morosus, Clitumnus extradentatus (Dupont Raabe, 1952) and in Oncopeltus fasciatus, (Johannson 1958).

As far as the other components of the reproductive system are concerned, these are also affected by the removal of the neurosecretory cells of the brain. Destruction of these cells results in a shrinkage of the spermatheca in the female and the accessory glands in the male.

The activity of the suboesophageal ganglion, thoracic glands, and the corpora cardiaca/allata in T. granarium is also restrained by the removal of the neurosecretory cells in the brain. This central control by the neurosecretory cells of the brain has been previously shown by many investigators, in different insects.

The deprivation of the neurosecretory cells in the brain resulting in arrest of growth, moult and atrophy of the reproductive system in T. granarium can be considered as a consequence of three possible processes. The first is that the neurosecretory cells secrete the hormone which directly causes the larva to moult and pupate and stimulates the ovarial and testicular development. The second is that the brain hormone stimulates the activity of the other endocrine centres to produce their hormones and consequently these physiological processes proceed. The third is that the secretion of these cells may be non specific, but enhance the general metabolism of the insect.

These three possibilities can be explained as follows:-

From the histological examination, confirmed by the autoradiographic study, and from the experimental work, there is evidence that the action of the brain neurosecretory cells in T. granarium and

their influence on the different physiological processes could be correlated with a specific action of their secretion. Growth, dormancy, ovarial and testicular development appear to be determined by the levels of concentration of the cells' hormone. The higher the rate of release the less liable the larva is to become dormant and the higher the achievement of ovarial and testicular development in the adult. In T. granarium the B. cells in particular seem to be involved in the control of diapause. Apart from the accumulation of the secretion within them, their number appears to increase during dormancy. Similarly, B-cells in Chilo suppressalis are completely inactive during diapause and become active before pupation, (Mitsuhashi and Fukaya 1960). Also, Siew (1963) observed differences in the histological appearance and the number of the B-cells in G. tanaceti during its imaginal diapause.

The restraint of the suboesophageal ganglion, the atrophy of the corpora cardiaca/allata and the inactivity of the prothoracic glands in T. granarium which has been deprived of its brain neurosecretory cells, provides evidence that the ganglion and these glands depend for their function on a stimulus from the brain. This stimulation may be either nervous or humoral. The brain control on the activity of other endocrine centres has been shown in many other insects. In Bombyx mori, the liberation of the suboesophageal ganglion hormone depends upon the acticity of the brain; this influence is exerted through the circumoesophageal commissures, (Fukuda 1951b, 1952, 1953c). Englemann (1957) has shown that the activity of the corpus allatum during ovarian development in Leucophaca maderae is regulated by purely nervous stimuli from the brain and the suboesophageal ganglion. Similarly, the atrophy of the corpora cardiaca/allata in the adult deprived of the neurosecretory cells of the brain has been shown in Calliphora (Thomsen, 1952), Oncopeltus (Johannson, 1958), Dermestes (Ladduwahetty, 1962) and in G. tanaceti (Siew, 1963). In the present work it has been shown that in the absence of the brain hormone, the thoracic glands did not secrete the moulting hormone, thus the larvae could not moult. This chief action of the brain hormone on the thoracic glands has been demonstrated excellently by Williams (1946, 1948, 1952) in Hyalophora.

Every endocrine gland affecting growth exerts its action by influencing metabolism (Wigglesworth 1954b). In Calliphora the neurosecretory cells in the brain stimulate the production of proteinase by the intestinal cells (Thomsen and Møller 1960) and they are also necessary for the

consumption of protein (Strangways, Dixon, 1961a,b, 1962). In Schistocerca gregaria blood protein concentration has been correlated with the activity of the neurosecretory system. The more active the neurosecretory system, the higher the concentration of protein in the haemolymph, (Highnam et al. 1963 a, b) and (Hill, 1963). Protein has been found to be necessary for egg growth. In T. granarium there is also a correlation between the metabolic rate and the activity of the neurosecretory cells. In dormant larva the rate of metabolism and food consumption are low whereas in non-dormant larva they become high (Burges, 1960). In the present work the histological examination of the neurosecretory cells in dormant and non-dormant larva and the autoradiographic study revealed that the cells are in a state of "low release" in dormant larva and in a state of "high release" in non-dormant larva. Therefore, metabolism in T. granarium is also affected by the activity of the neurosecretory cells. The higher rate of release the greater the metabolic rate.

This hormonal control of metabolism is either a direct control from the brain hormones or indirect by restraining the activity of other centres such as the corpus allatum. The metabolic effects of the corpus allatum hormone in growth will be discussed later.

2. The suboesophageal ganglion and the control of metamorphosis and reproduction:

The part played by the neurosecretory cells of the suboesophageal ganglion of T. granarium in metamorphosis and reproduction is apparently similar to the role of the brain cells in controlling these physiological processes.

The histological examination of the cell during dormancy, growth, ovarial and testicular development, reveals a definite pattern of accumulation and release of its secretion. Dormant larva, unmated female, and male, and the female which had entered a post-oviposition stage had their cell in a state of "low release". Whereas in non-dormant larvae, mated male and in ovipositing female, the cells were in a state of "high release".

Cauterisation of the suboesophageal neurosecretory cells in non-dormant larva prevented it from moulting and pupating in pupa extirpation of the cell inhibit adult formation; and in dormant larva cauterisation impeded dormancy. In the adult removal of the neurosecretory cell retarded egg maturation and inhibited oviposition in the female; and in the male

it also retarded spermatogenesis and caused a shrinkage in the accessory glands. Therefore, all these physiological processes appear to be determined by a level of concentration of the suboesophageal ganglion hormone circulating in the blood.

The importance of levels of concentration of hormones from the various endocrine centres and the differential sensitivities of the target organs has been noted in several insects; e.g. Rhodnius (Wigglesworth, 1936), Melanoplus differentialis, (Pfeiffer, 1945), Calliphora (Thomsen 1952), Leucophaea maderae and Oncopeltus (Johannson, 1955, 1958).

Histological examinations of T. granarium deprived from its suboesophageal neurosecretory cell revealed that the brain neurosecretory cells reverted to a low rate of release and the corpora cardiaca/allata became inactive. This suggests a feed-back mechanism to the brain and an influential control on the corpora cardiaca/allata. The feed-back mechanism could be brought about in two ways; the first is directly to the brain via the circumoesophageal commissures and the second way is via the corpus cardiacum-allatum-suboesophageal nerve to the corpus cardiacum/allatum, in turn to the brain. A feed-back mechanism from the corpora allata to the neurosecretory cells of the brain in Calliphora has been experimentally demonstrated (Lea and Thomsen, 1962).

The suboesophageal secretion appears to be necessary for the continuous activity of the corpora cardiaca/allata in T. granarium. Experimentally, it has been shown that cauterisation of the neurosecretory cell of the ganglion prevents the corpus allatum from secreting its yolk formation and accessory glands activating hormones. The corpus allatum/cardiacum suboesophageal nerve exists in T. granarium. So it is tempting to suggest that the cell may have a stimulatory effect on the activity of the corpus cardiacum and allatum directly via this nerve or indirectly via the brain in turn to the corpora cardiaca allata. Englemann (1957) has experimental evidence that the suboesophageal ganglion of Leucophaea maderae stimulates the activity of the corpus allatum via nervous impulses. Harker's (1960c) observations on Periplaneta americana, on the other hand, show that the direction of the axonal transport of neurosecretory material is from the corpus cardiacum to the suboesophageal ganglion. The subtle relationships between the various endocrine centres have yet to be resolved.

In other insects the suboesophageal ganglion produces hormone which controls the copulatory reflexes, cockroaches (Roeder, 1935), controls voltinism, Bombyx mori, (Fukuda, 1951, 1952, 1953); controls a 24 hour rhythm of activity, Periplaneta americana (Harker 1956, 1958, 1960) and influences colour change in phasmids, (Possompès, 1957). In G. tanaceti, cauterisation of the suboesophageal neurosecretory cells, retarded oviposition and caused the imaginal diapause, though implartation of the ganglion did not terminate diapause (Siew, 1963). In all these insects, the suboesophageal ganglion produces its hormone stimulated by the brain hormone, but the humoral activity of the brain appears to depend on an uninterrupted nervous connection between brain and the suboesophageal ganglion.

3. The role of the corpora cardiaca in metamorphosis and reproduction

The functional significance of the corpus cardiacum has not been fully understood. In T. granarium the corpus cardiacum has been shown to function as a storage organ for the brain neurosecretion. Similarly the corpus cardiacum is a storage organ in Calliphora (Thomsen E. 1952, Herlant Meewis and Paquet, 1956) and in Leucophaea maderae (Scharrer 1951, 1952). Conversely, the gland does not act as a storage organ in Hydrocirius (Junqua, 1956). Oncopeltus (Johannson, 1958) and in Dermestes (Ladduwahetty, 1962).

In many insects the corpora cardiaca act as neurohaemal organs (Van der Kloot, 1960). There is evidence that they discharge the secretions which accumulate in them directly into the blood. Similarly, in S. gregaria the neurosecretions from the brain accumulate in the corpora cardiaca which constitute a great part of the ventral wall of the aorta; the secretions are discharged through this wall to the blood (Highnam, 1961). In T. granarium the corpora cardiaca are intimately close to the aorta. In some specimens the ventral wall of thegland and the dorsal wall of the aorta appear to be one single wall. It is tempting to speculate here that the corpora cardiaca may release the secretions into the aorta through this part.

There is some evidence from the measurements of the volumes and the histological structures of the corpus cardiacum throughout the definite intervals of the life-history of T. granarium that this gland undergoes cycles of activity. These cycles of activity have been shown to fluctuate intensely during any one major physiological phase. They tend to intensify during the phases of moulting, ovariole maturation and moreso towards and during the

phases of pupation and oviposition, suggesting a rôle of this gland in these processes.

The functional significance of the secretion of the corpus cardiacum in T. granarium could not be demonstrated experimentally as extirpation or implantation of the gland has been found impossible. Therefore, the function of the secretion of the gland can only be postulated in the light of the findings of previous investigators as follows:-

(a) the secretion itself may have an effect on other centres.

(b) or may be mixed with the other secretion from the brain before the hormones are discharged from the gland;

(c) and finally the secretion may merely act as a "catalyst" for the brain "hormones".

The gland's extracts have been shown to stimulate contractions of gut muscles and malpighian tubules (Cameron, 1953). Wigglesworth (1954) stated that the corpus cardiacum might be concerned in the secretion, not of growth hormones but of a pharmacologically active principle or principles comparable with those produced by the pituitary or supravenal glands of vertebrates. The corpus cardiacum in Rhodnius contains heart activating substance (Wigglesworth 1954). In cockroaches the secretion from the corpus cardiacum has been shown to stimulate the pericardial cells and to produce a pharmacologically active agent (Davey, 1961, 1962). The corpus cardiacum extract stimulates then depresses nervous activity (Ozbas and Hodgson, 1958), Milburn et al. (1960). The corpus cardiacum isalso thought to inhibit the activity of the inhibitory centre in the suboesophageal ganglion in Periplaneta americana (Milburn et al, 1960).

The corpora cardiaca and the brain together are necessary for full ovarial development in Schistocerca gregaria; cautery of the neurosecretory cells or removal of the corpora cardiaca retards the development of the terminal oocytes (Highnam, 1962). Removal of brain and corpora cardiaca has been found to cause disturbances in protein metabolism in Calliphora (Thomsen, 1952).

Finally, it has been suggested that the brain hormone may be a labile raw material from which hormones manufactured by different parts of the endocrine system may be derived, (Wigglesworth, 1954b). Consequently, it is reasonable to deduce that the secretion of the corpora cardiaca in T. granarium may be a catalyst for the labile raw materials which are secreted by the brain.

Nevertheless, the activity of the corpora cardiaca in all insects has been shown to depend upon stimulation from both brain and the suboesophageal ganglion.

4. The corpora allata and the control of metamorphosis and reproduction.

The part played by the hormone of the corpora allata in T. granarium, in metamorphosis and reproduction is not less important than the part played by the neurosecretory cells in controlling these physiological processes.

The volume of the corpus allatum has often been referred to as a standard of its activity. Calculations of the volume of corpus allatum in T. granarium, throughout different physiological phases, have definitely indicated that these processes are under a control of the corpus allatum. Experiments have also shown that the corpus allatum hormone is responsible for metamorphosis, yolk formation and for the accessory glands to produce their secretion. This is in agreement with the findings of previous workers. The function of the corpus allatum in controlling metamorphosis, by causing the insect to retain its larval or juvenile character when it moults has been confirmed in many insects from various orders, (See Wigglesworth, 1954a). The corpus allatum and its control on yolk deposition have also been demonstrated in Rhodnius (Wigglesworth 1936, 1961), Locusta migratoria (Joly, 1960), Dermestes, (Ladduwahetty, 1962), Schistocerca gregoria, (Highnam, 1963) and in many other insects, see (Wigglesworth, 1954a and 1964). In the male of Rhodnius (Wigglesworth, 1936) and Leucophaea (Scharrer, 1946b), the corpus allatum is also necessary for the accessory gland to become filled with secretion. On the other hand, egg maturation proceeds without interruption in allatectomized Carausius morosus, unless the glands are extirpated at an early nymphal stage (Pflugfelder, 1937a,b, 1939). Similarly, the corpora allata are apparently not necessary for egg production in certain Lepidoptera, e.g. Bombyx mori (Fukuda, 1944). However, in these cases, the developing pupae have fully mature eggs and probably there is still a high level of the corpus allatum hormone in the body (see Williams, 1956a).

The corpus allatum hormone in T. granarium may directly control metamorphosis and reproduction or indirectly by affecting the metobolic processes in the insects. This is inferred from the experimental evidence of Thomsen (1949), Thomson and Hamburger (1955) in Calliphora that allectomy reduced oxygen uptake while implantation of active corpora allata increased

oxygen uptake. Comparable results have been obtained in Pyrrhocoris opterus (Novak et al., 1960) and in Leptinotarsa decemlineata (de Wilde and Stegwee, 1958, de Wilde 1960). Pfieffer (1945a) had found that the corpora allata control egg production in Melanoplus through the agency of a metabolic hormone. Strangways-Dixon (1961a,b) has showed experimentally that the corpus allatum controls carbohydrate ingestion in Calliphora.

From the foregoing discussion it would appear that growth, metamorphosis and reproduction are merely under the control of a specific hormone produced by any component of the neuro-endocrine system. However, the results obtained in the present work indicate that this was an over simplification. It is not only the presence or absence of a hormone which prevents or induces any of these physiological processes, but it is the concentration and time of production that are important. Hormonal release has been found to be precise in quantity and time, otherwise it may cause profound pathological and physiological abnormalities.

The histological examination of the neurosecretory system in all stages of T. granarium during different physiological processes revealed a definite pattern of accumulation and release of the neurosecretory cells secretion. Larvae whose growth was arrested, and unmated females and males had their neurosecretory cells in a state of "low release". In this condition the cells are full of accumulated secretion and little product is transported along the axons. Whereas, in moulting larvae, ovipositing females and mated males the neurosecretory cells were in a state of a "high release". Here the cells contain few aggregates, but the axons are packed with neurosecretion. Therefore, moulting, oviposition and spermatogenesis appear to be determined by the concentration of the neurosecretory cells hormone circulating in the haemolymph or carried along pathways, i.e. a low concentration of hormone is sufficient for growth, complete maturation, but a high concentration of hormone is necessary for moulting, metamorphosis and ovulation of mature eggs.

Experimentally, it has also been shown that a high concentration of juvenile hormone circulating in the blood by applying farnesol on growing larva lowered the concentration of the brain hormone and consequently the larva did not pupate but retained its juvenile characters. Also the delay of egg and testicular development in the adult whose suboesophageal ganglion was cauterised indicates that a certain level of hormone is necessary for full maturation, oviposition and spermatogenesis.

Similarly, when the larva was treated with insecticides, the neurosecretory system became inactive and at low rate of release, thus metamorphosis, egg development and spermatogenesis were delayed.

The importance of concentration of hormones from the various components of the endocrine system and the differential sensitivities of the target organs have been noted in several insects, e.g. in Rhodnius, the different nymphal stages are determined by the levels of the juvenile hormone from the corpora allata circulating in the blood (Wigglesworth 1936, 1954). Parabiosis experiments in Rhodnius occasionally resulted in the development of only 2 - 3 ripe eggs despite the presence of oocytes in all the 14 ovarioles. Wigglesworth (1948) attributes this to great dilution of the hormone. Similar action of different hormone concentrations has been noted in Bombyx (Fukuda, 1944), Sialis (Rahm, 1952), Leucophaea (Johannson, 1955) and Oncopeltus (Johannson 1958).

Finally it must be mentioned that for each hormone there exists a certain period at which a high concentration would be most effective. At other periods this concentration could disturb the hormone balance, thus resulting in abnormalities and deformaties.

Farnesol, has been shown in T. granarium to have similar properties to juvenile and yolk formation hormone. When the larva was smeared with farnesol, it did not pupate and remained in the larval stage for a longer period. When a young pupa was smeared with farnesol, metamorphosis was inhibited and the pupa never became adult. Also when a newly emerged adult was treated with farnesol after its suboesophageal ganglion neurosecretory cell was removed, yolk formation occured in the female and the accessory gland of the male became filled with secretion. However, when an older pupa was smeared, different grades of deformation and abnormalities occured.

Similarly, when larvae of T. granarium were treated with insecticides some developed to deformed pupae or adults as a result of upsetting the concentration and timing of the secretion from the neuroendocrine system.

Similar abnormalities resulted from some upset in the balance of hormones during development have been shown in other insects by previous workers, e.g. Tenebrio (Pruthi, 1924), and(Radtke, 1942), Rhodnius (Wigglesworth, 1934, 1936), Gryllus (Sellier, 1951) and quite recently in Pyrochroa coccinea (Lessmann, 1964).

Upset in the balance of hormones leads, sometimes, to pathological conditions or even to paralysis, and death. In the present work, it has been shown that some larvae, after treatments with insecticides, became paralysed. Surgical severance of the recurrent nerve of Leucophaea maderae resulted in midgut tumors, (Scharser, 1945). Similar midgut tumors, have been shown in Periplaneta americana, by upsetting the timing and concentration of the secretion from the suboesophageal ganglion (Harker, 1958).

The overall picture of endocrine functions in Trogoderma granarium appears to be essentially the same as in other insects. Attention might, however, be drawn to the ontogony of the neurosecretory cells in particular the B-cells appearing, for the first time, in the fourth-instar larva, and the C-cells in the last larval instar which are absent in the pupa and adult. With the consistent cytological appearance and stain affinity of the different cell types, it is not unreasonable to assume a "division of labour" among them. This hypothesis is supported in part by the experimental ablation of the C-cells and the ensuing physiological consequences. The establishment of the precise roles of the endocrine centres in the central nervous system lies in the future, and will probably only be achieved with isolation and purification of the active substances involved. Only in this way can the affects of single cell types be assessed in terms of individual tissue response and target organs.

SUMMARY

1. The central nervous system in Trogoderma granarium evidently grows by a combination of increase in cell size and in cell number.

2. Neurosecretory cells have been found in the brain and the suboesophageal ganglion in the larvae, pupae and adults. None has been found in any of the other ganglia.

3. Two groups, median and lateral groups, of neurosecretory cells have been located in all stages except the first and second instar larvae, where there are only median groups.

4. Three types of cells, A, B & C-cells, have been found. B-cells have been only found in the median group and the C-cells are only found in the suboesophageal ganglion of the mature larvae.

5. The neurosecretory cells in the larvae exhibit different levels of activity, determined by the external environment. Food and temperature play a great role in the synthesis and release of hormones of the neurosecretory system. Temperature has been shown to have a direct action on the release of the neurosecretory products.

6. The histological observations were substantiated by the use of a radioactively labelled amino acid S^{35}-D-L-cystine in autoradiographic studies. The neurosecretory cells in non-dormant larvae have been found to be twice as active as those of dormant larvae.

7. Correlated with these changes in the level of activity of the neurosecretory cells, the corpora cardiaca/allata follow a similar pattern in changes of activity.

8. The activity of the neurosecretory system in the pupa varies throughout the pupal life. The secretory activity increases towards the pupal-adult moult period.

9. The anatomy, histology and growth of the reproductive system in both male and female have been studied.

10. The histological examination of the neuro-endocrine system of mated and unmated adults of T. granarium revealed a definite pattern of accumulation and release of secretory material, associated with the different physiological phases. The activity of the neurosecretory system reaches its peak during the oviposition period.

11. There is no conspicuous increase in the release of neuro-secretion material in the female during copulation, whereas in the male the

rate of release is higher than normal.

12. The brain neurosecretory cells exert an overall controlling influence on growth, metamorphosis, maturation, oviposition and spermatogenesis.

13. Extirpation of the neurosecretory cells of the brain:

(a) inhibited the larval-pupa moult in non-dorman larva;

(b) impeded the termination of dormancy in dormant larvae irrespective of the stimuli of the environment;

(c) hindered the pupa-adult moult; and finally,

(d) prevented copulation, ovarial and testicular development and oviposition.

14. Neurosecretory cells of the suboesophageal ganglion do not initiate moulting nor ovarial and testicular development but their secretion has been found to be necessary for the physiological processes to proceed.

15. Farnesol, simulating the hormones secreted by the corpus allatum, causes retention of the larval characters, delays eclosion of the adult, induces yolk deposition in females and activates the accessory glands to secrete their secretion.

16. Disturbance of the hormone balance leads to the development of forms intermediate between those of larvae and adults.

17. Fresh spermatozoa increase the number of eggs laid by females. The period of "survival" of the spermatozoa in the spermatheca is approximately two days.

18. Certain insecticidal treatments lead to the hindrance of the neurosecretory system and provide further evidence that hormonal release must be precise in quantity and time otherwise it may cause profound physiological consequences.

ACKNOWLEDGEMENTS

I am greatly indebted to Professor O.W. Richards, F.R.S., for accommodating me at the College Field Station, and for providing me with research facilities.

I also wish to thank Dr. A.B.P. Page for his invaluable assistance and Professor B.G. Peters for occasional use of his equipment.

I am most grateful to my Supervisor of Study, Dr. C.T. Lewis, for his invaluable help and encouragement and to Mr. R.G. Davies for his constructive criticisms and advice and for translating some German papers.

I am thankful to Dr. H.D. Burges of the Pest Infestation Laboratory for providing me with the dormant larvae.

Thanks are also due to Mr. M.J. Way for his interesting discussion, to Mr. J.W. Siddorn for help with photography and Mr. G. Murdie for help in Statistics.

I am thankful to Mrs. M. van Emden for translating some German papers and for help with references, Mrs. A. Nadim, Mrs. B. Murdie and Mrs. T. Sarney for help with preparing the manuscript.

This research was carried out during the tenure of a study leave awarded by the Government of the United Arab Republic. I am thankful to the Directors of United Arab Education Bureau of London for their support.

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Fig. I: The central nervous system in the mature larva.

1,2, and 3: pro, meso, and metathoracic segments;
1 - 1X: abdominal segments;
Br: brain;
H: head;
S.oe.g: suboesophageal ganglion.

Fig. 1

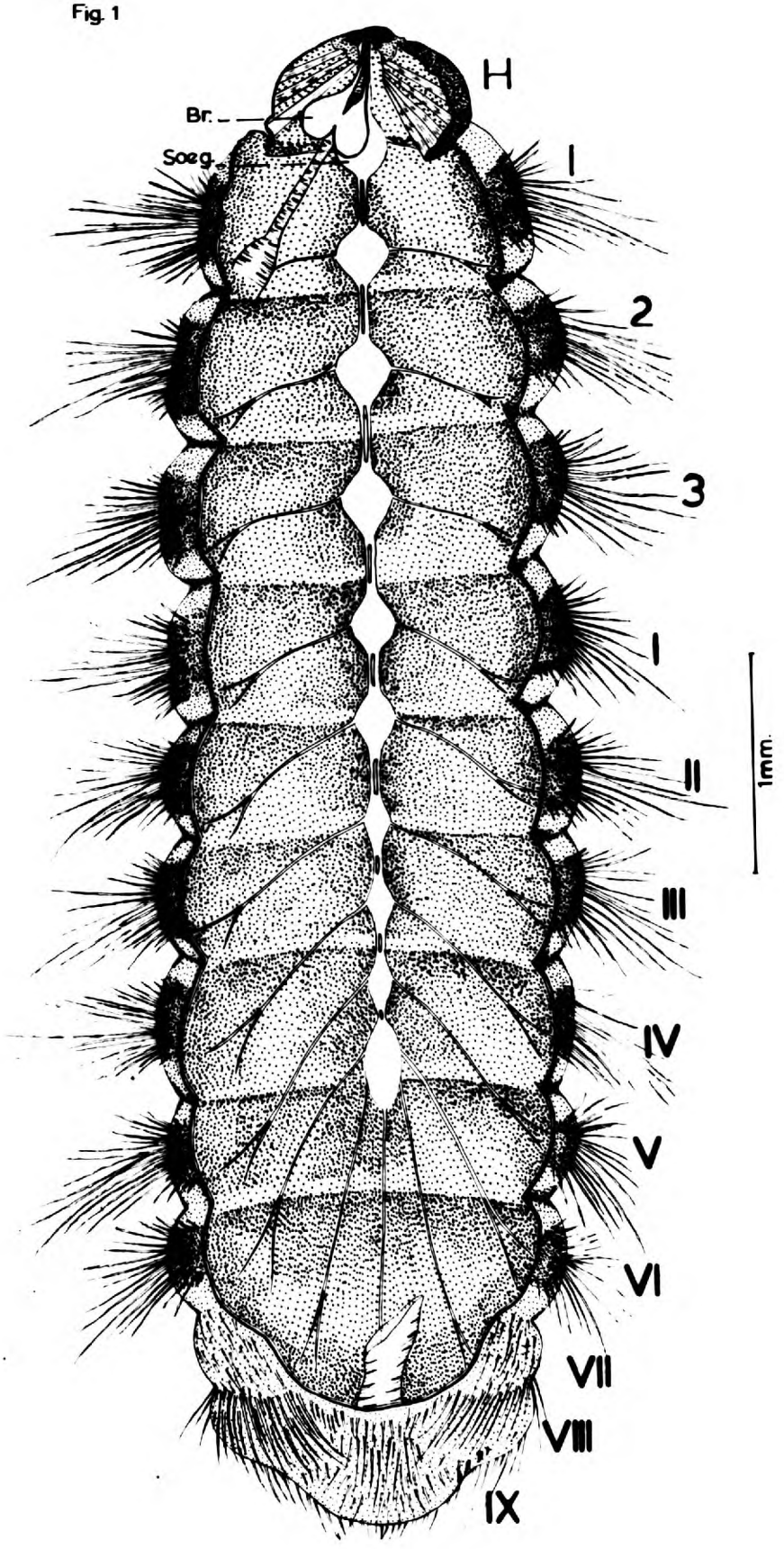
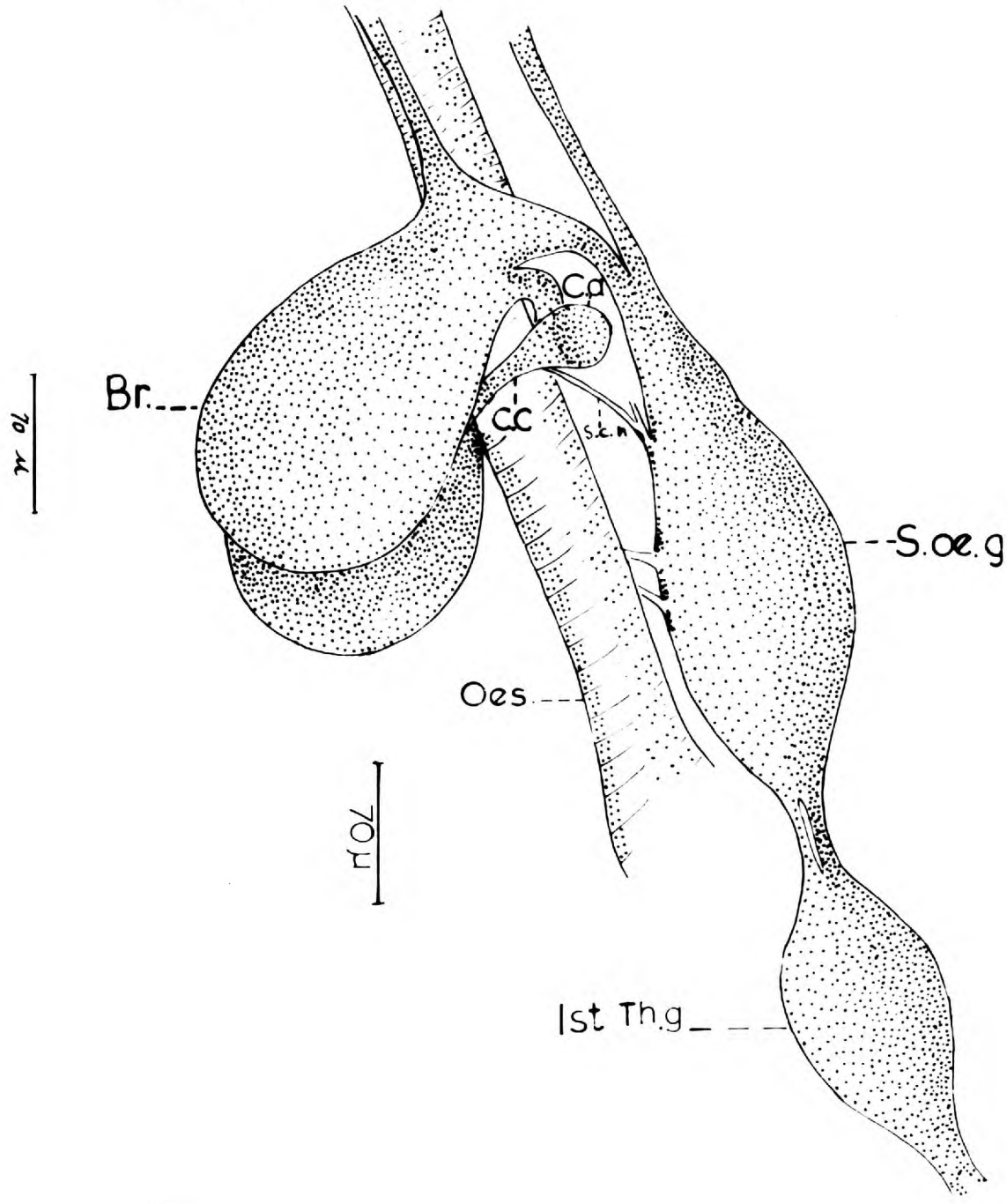


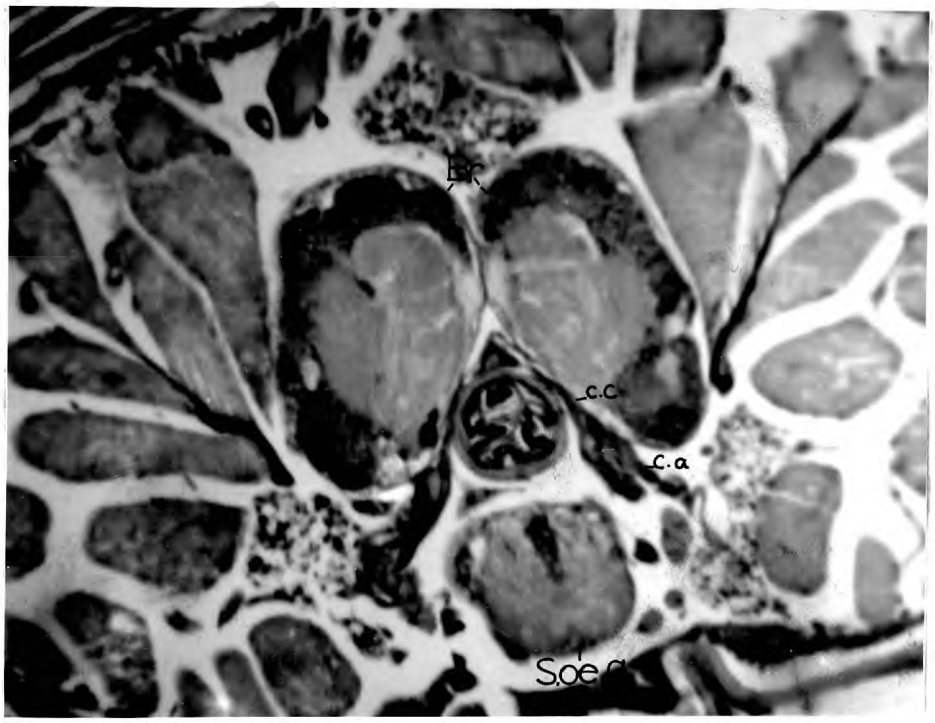
Fig.2a: Lateral aspect of the neuro-endocrine system in the larva.

Br: brain;
Ca: corpus allatum;
Cc: corpus cardiacum;
Oes: oesophagus;
s.c.n: suboesophageal-corpara nerve;
S.oe.g: suboesophageal ganglion;

1st.Th.g: first thoracic ganglion.

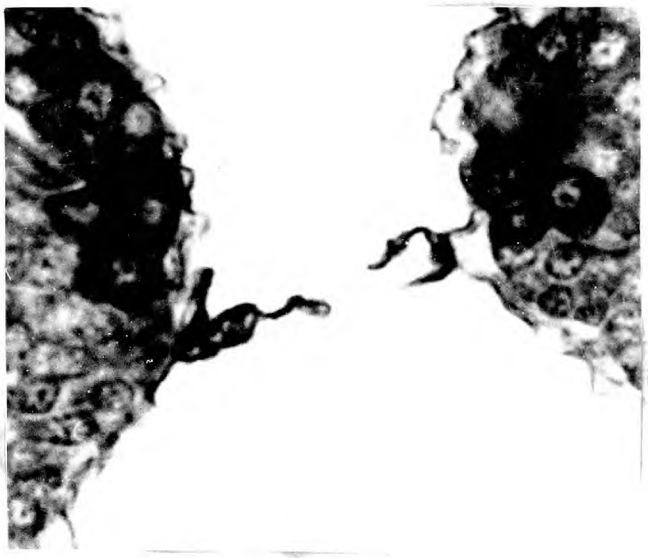
Fig. 2 a.





215 u

Fig. 2b: Across section through the endocrine system of mature larva. Explanation of lettering on previous page (Fig.2a.)



18 u

Fig.3: The two "median groups" of neurosecretory cells in 1st-instar larva.

Fig. 2c

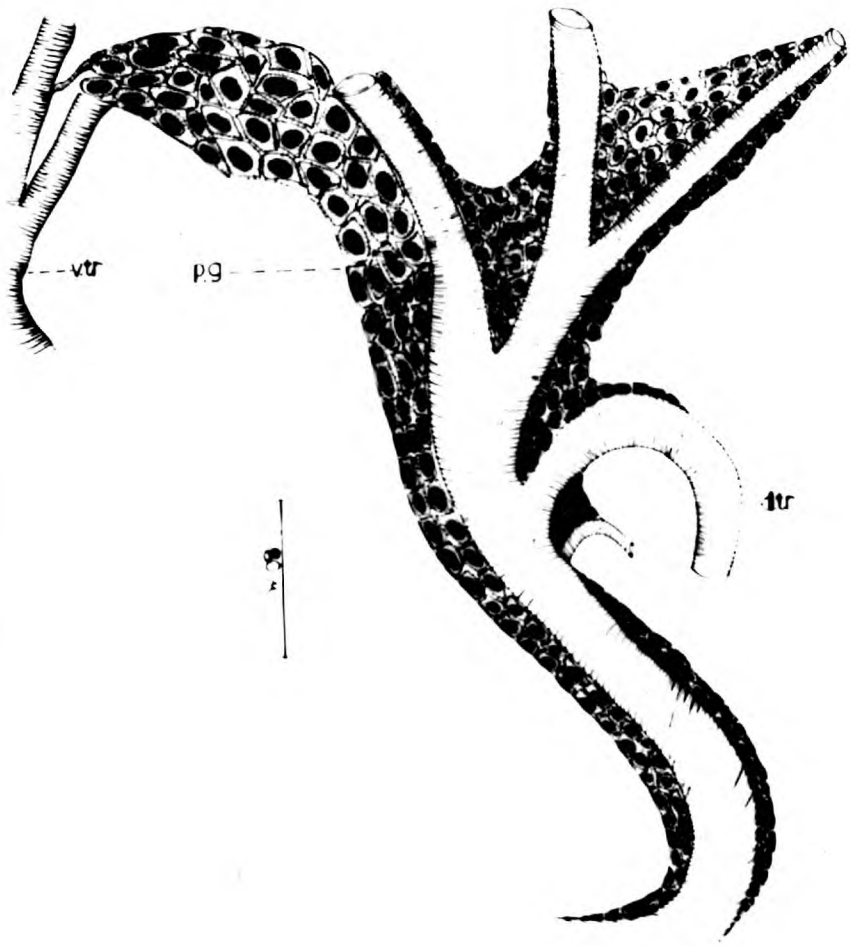


Fig.2c: The prothoracic glands in mature larva.
d.tr: dorsal trachea; p.g: prothoracic gland;
v.tr: ventral trachea.

Fig. 4.

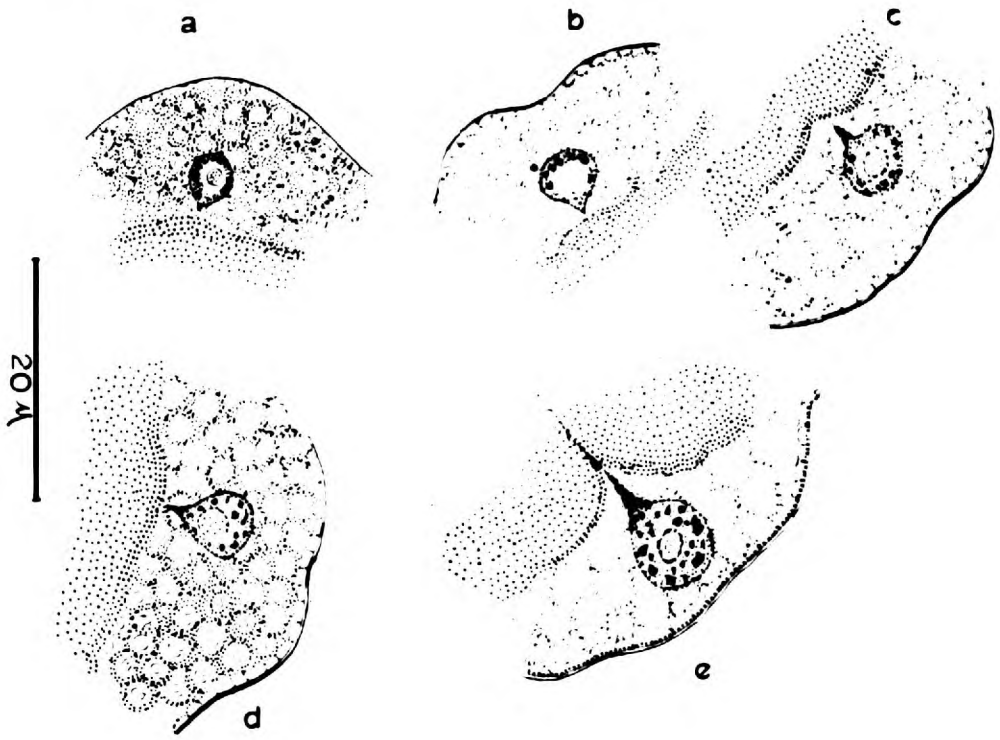
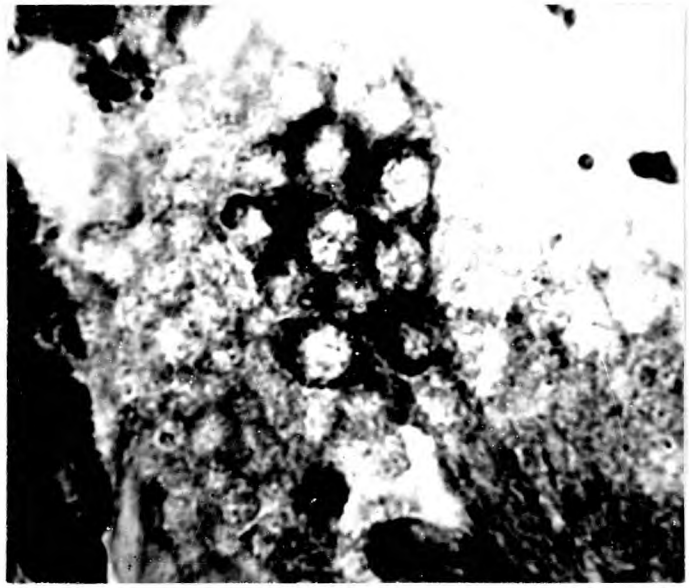
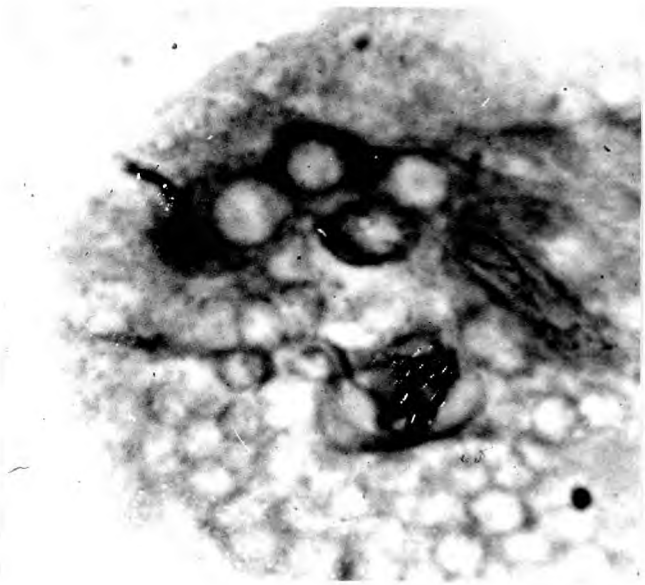


Fig. 4 a-e: A-cells of the suboesophageal ganglion in 1st- ,2nd- ,3rd- ,4th- and sixth instar larvae respectively.



10 u.

Fig.5: Neurosecretory cells in the brain of the 2nd-instar larva.



10 u.

Fig.6: Neurosecretory cells in the brain of the 3rd-instar larva.

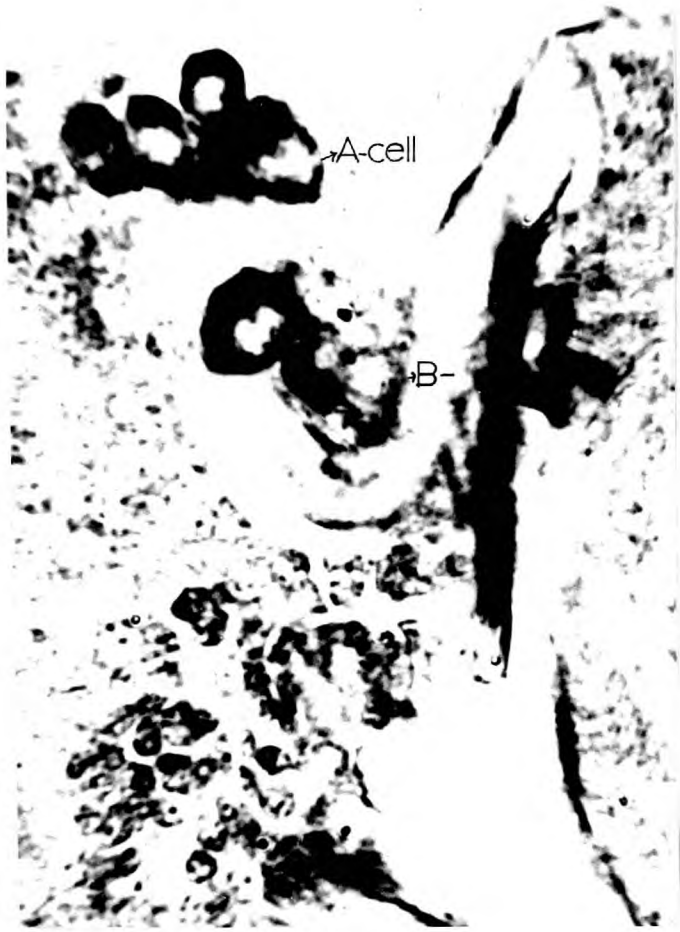
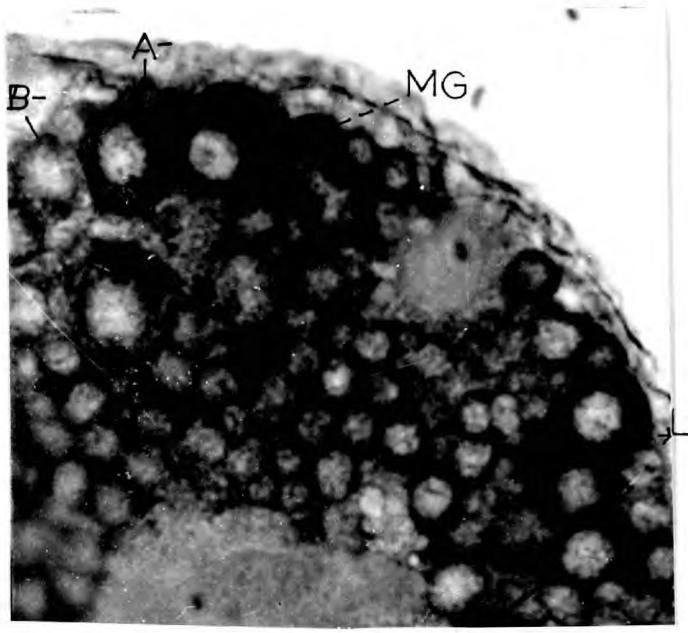


Fig.7: Neurosecretory cells , medial groups, in the brain of fourth instar larva.

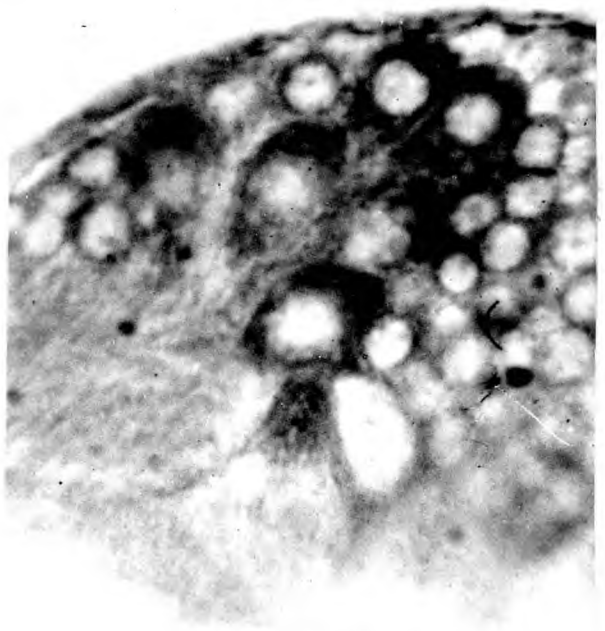
(A)



20 u.

Medial and lateral groups.

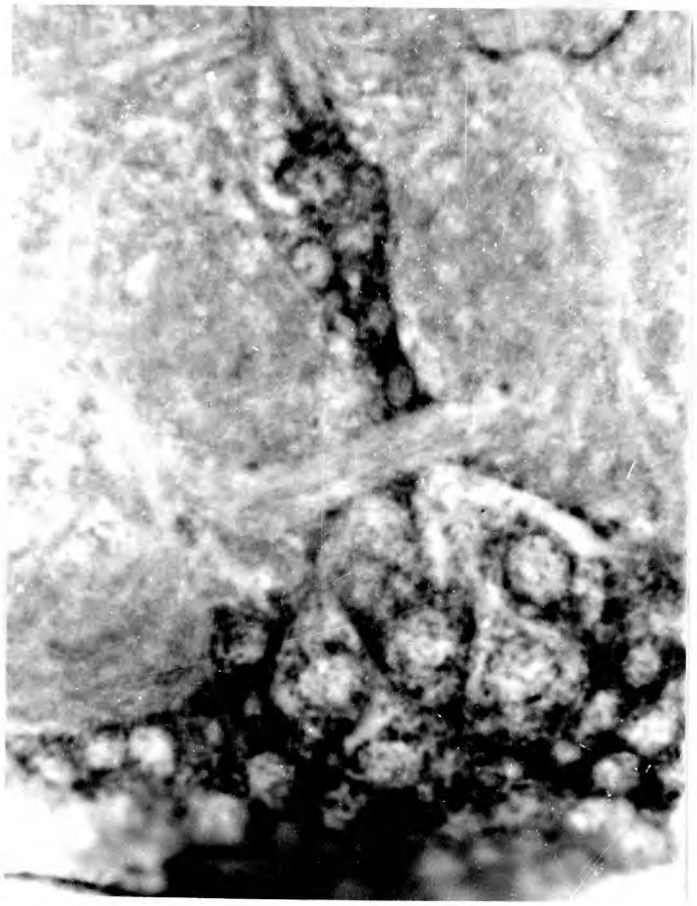
(B)



20 u.

Medial groups.

Fig.8: Neurosecretory cells in the brain of mature larva.
(A) Cells in larva fixed during the intermoult period.
(B) Cells in larva fixed during moulting.



13 u.

Fig.9. A cross section through the suboesophageal ganglion showing the C-cells.

Fig. 10.

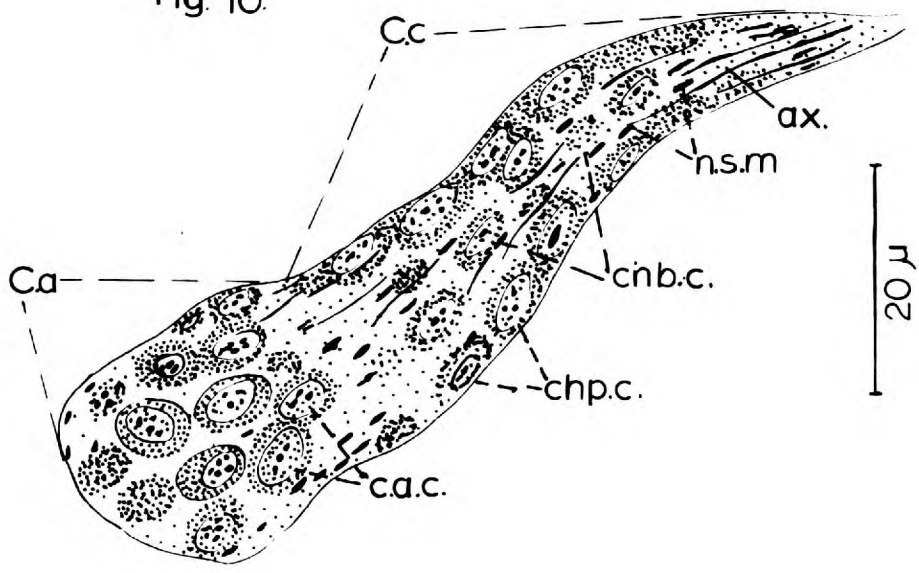
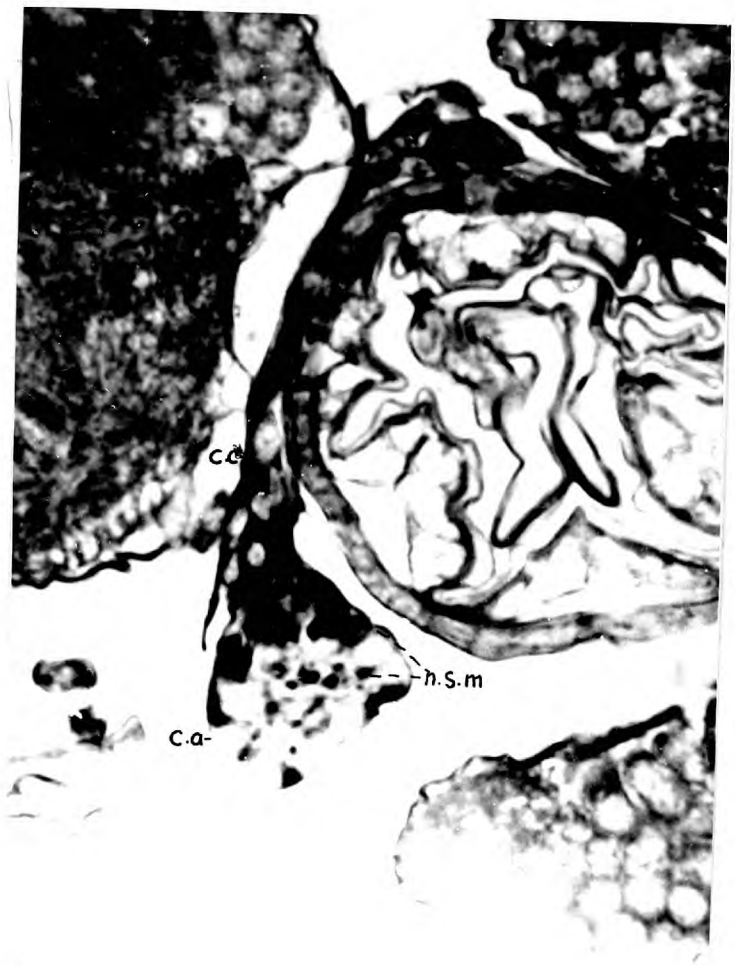


Fig.10: Corpus cardiacum and allatum in the larva.

C.a: corpus allatum;
C.c: corpus cardiacum;
ax : axons
c.a.c: corpus allatum cells;
chb.c: chromophobe cells;
chp.c : chromophile cells;
n.s.m: neurosecretory material.



25 u.

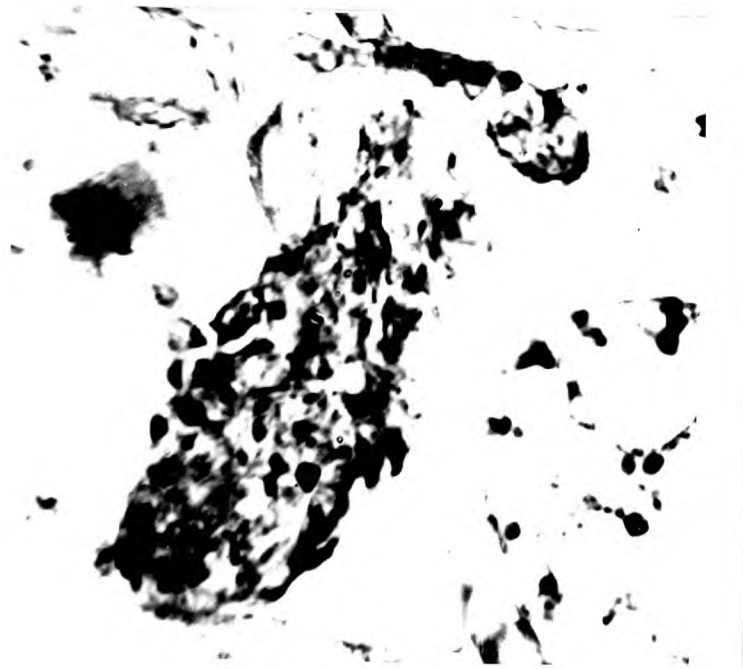
Fig.11: The corpus cardiacum and allatum in 1st-instar larva. For explanation of lettering see fig.10.

18 u.

Fig.12: The corpus cardiacum and allatum in 2nd-instar larva. Notice the dark stained substance(S); c.f. page 26.

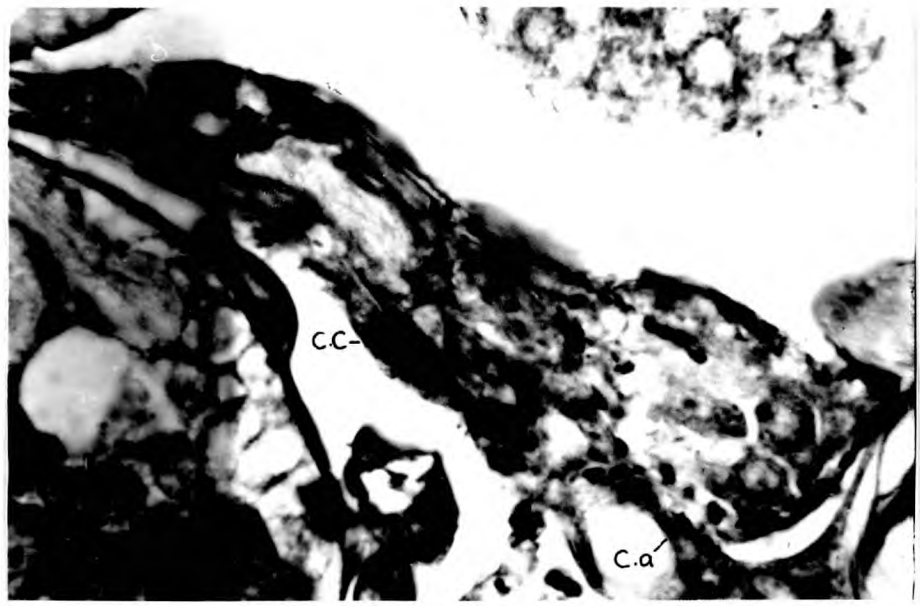
20 u.

Fig.13: Corpus allatum(Ca) and corpus cardiacum (Cc) in the third instar larva. For details see fig.10.



20 u.

Fig.14: The corpus cardiacum and allatum in 4th-instar larva.



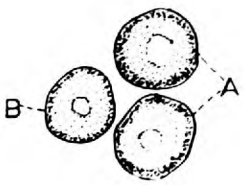
25 u.

Fig.15: The corpus cardiacum(Cc)and allatum(Ca) in mature larva.
For detailes see fig. 10.

Fig.16: A drawing of different stages of neurosecretory cells in larva T. granarium in different environments.

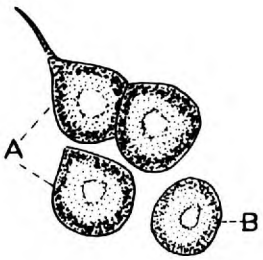
A: A-cell;
B: B-cell.

Fig. 16



I

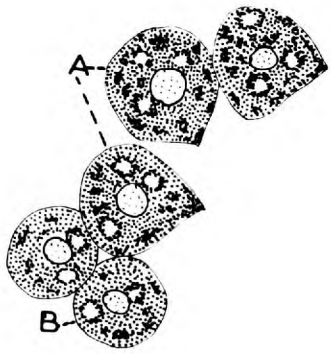
VIII



II

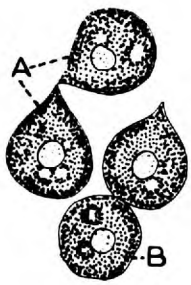
VII

20 μ

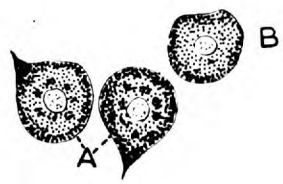


III

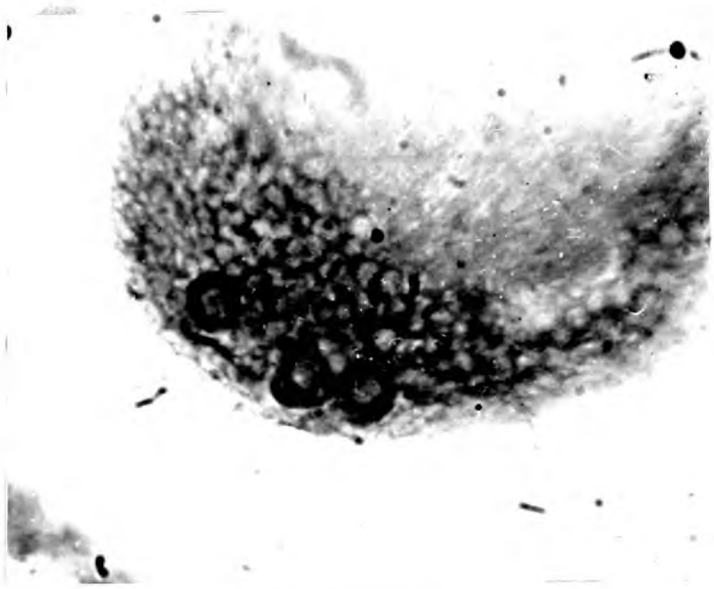
VI



IV

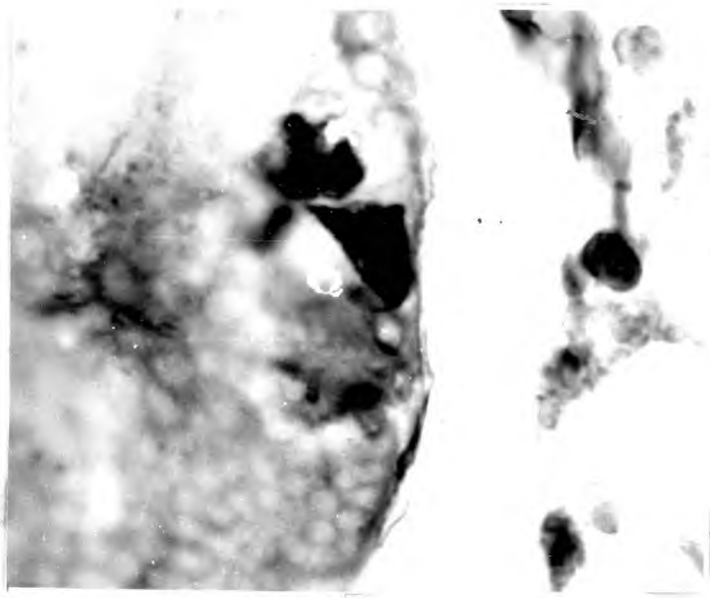


V



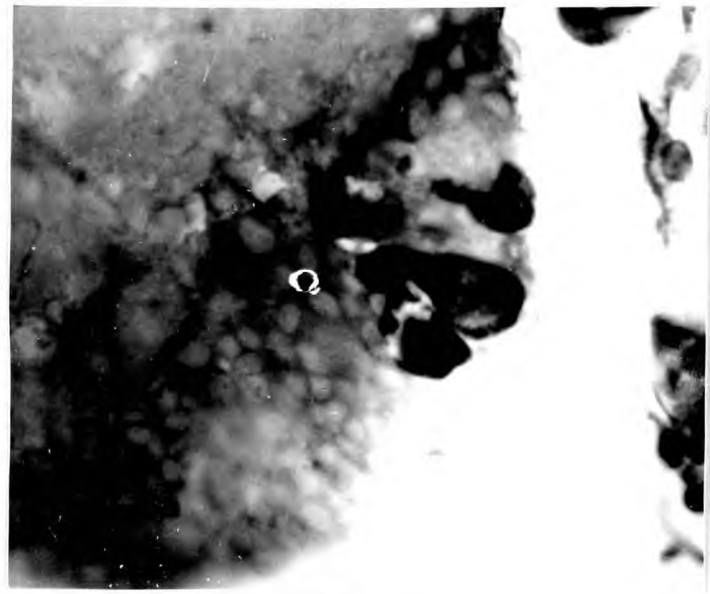
20 u.

Fig.17: Neurosecrtory cells in larva after two weeks in dormancy. Kept at 25 C and with food.



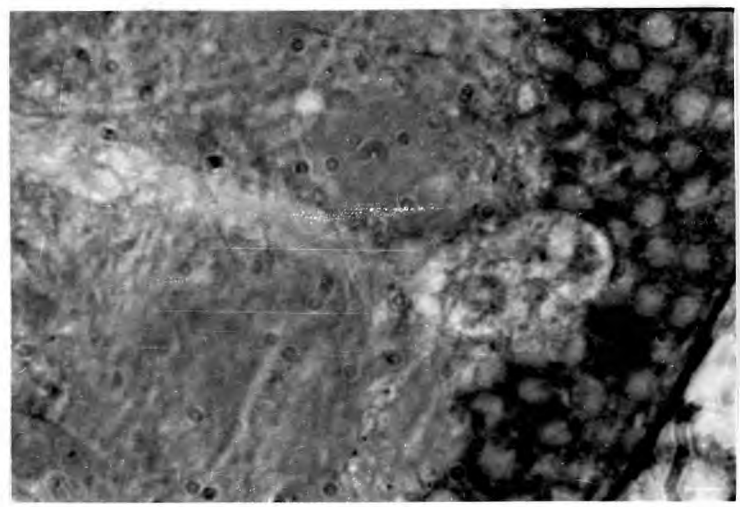
10 u.

Fig.18: Neurosecretory cells in larva after 8 weeks in dormancy. Kept at 25 C and with food.



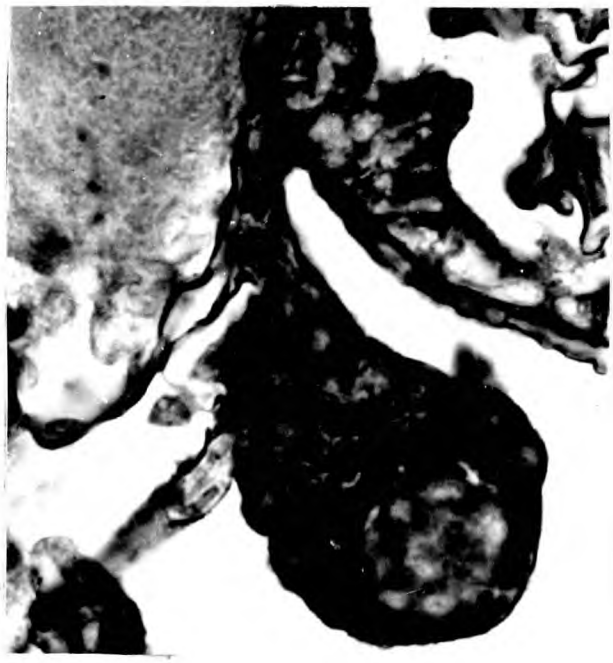
10 u.

Fig.19: Neurosecretory cells in larva after ten weeks in dormancy. Kept at 25 C and with food.



20 u.

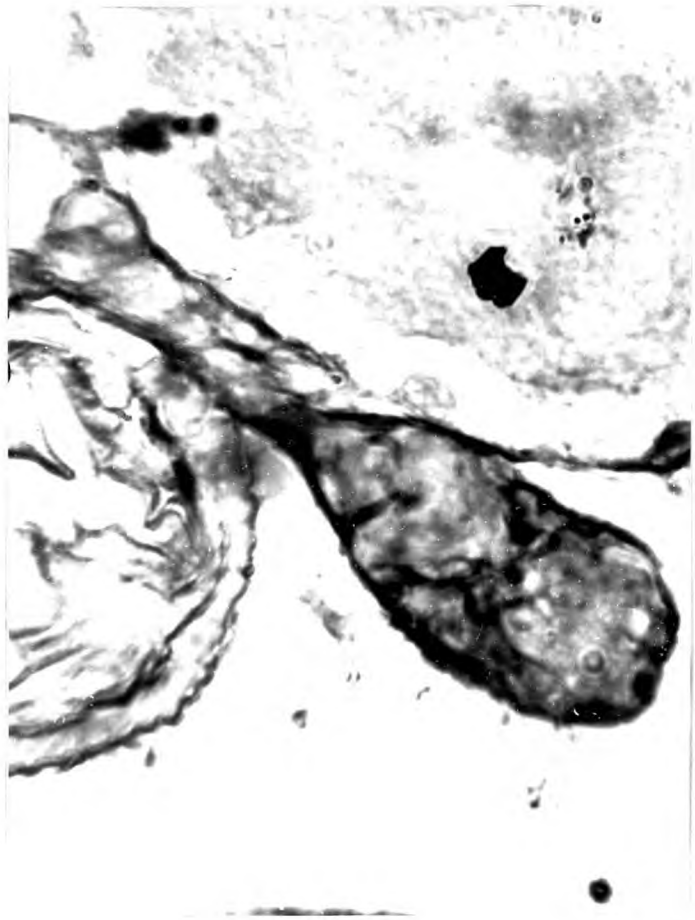
Fig.20: C-cells in larva after ten weeks in dormancy. Kept at 25 C and with food.



15 u.

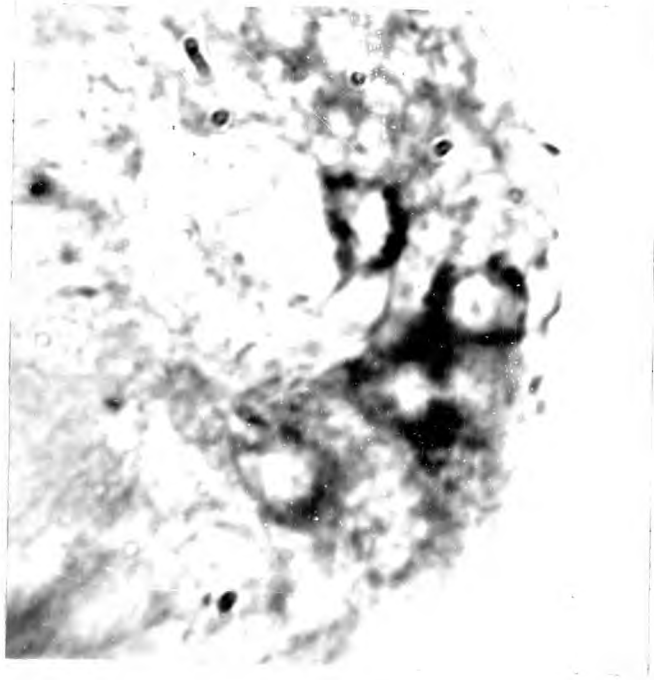
Fig.21: The corpus cardiacum(Cc) and corpus allatum(Ca) in larva after ten weeks in dorma[illegible].

Fig.22: A cells in larva after six weeks in dormancy. Kept at 25 C and without food.



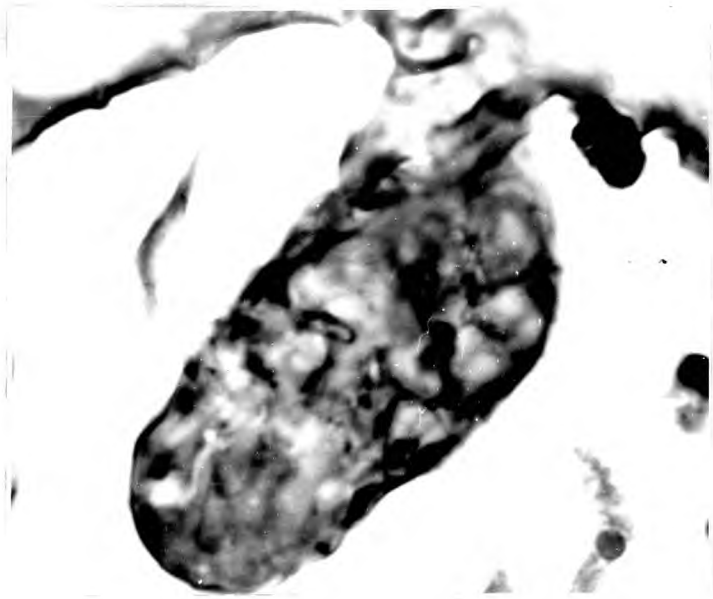
20 u.

Fig.23: The corpus cardiacum(Cc) and corpus allatum(Ca)in larva after six weeks in dormancy. Kept at 25 C and without food.



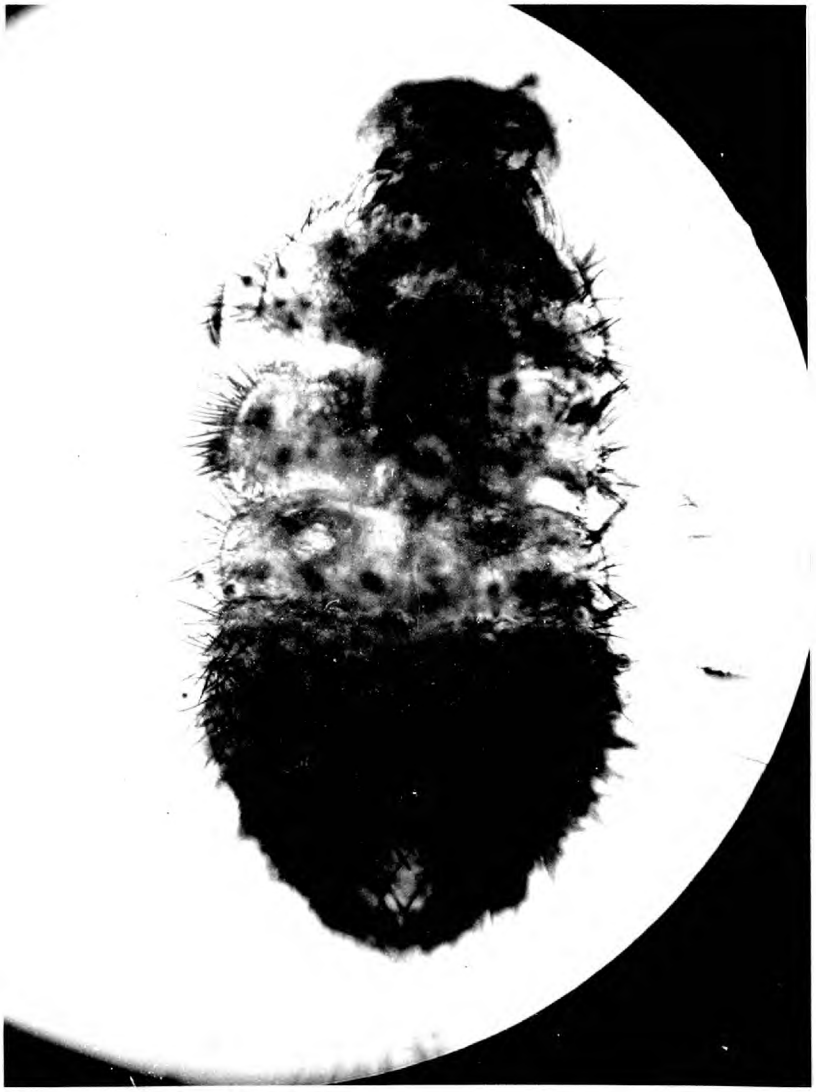
15 u.

Fig 24: **A** - cells in larva after four weeks in dormancy. Kept at 30 C and without food.



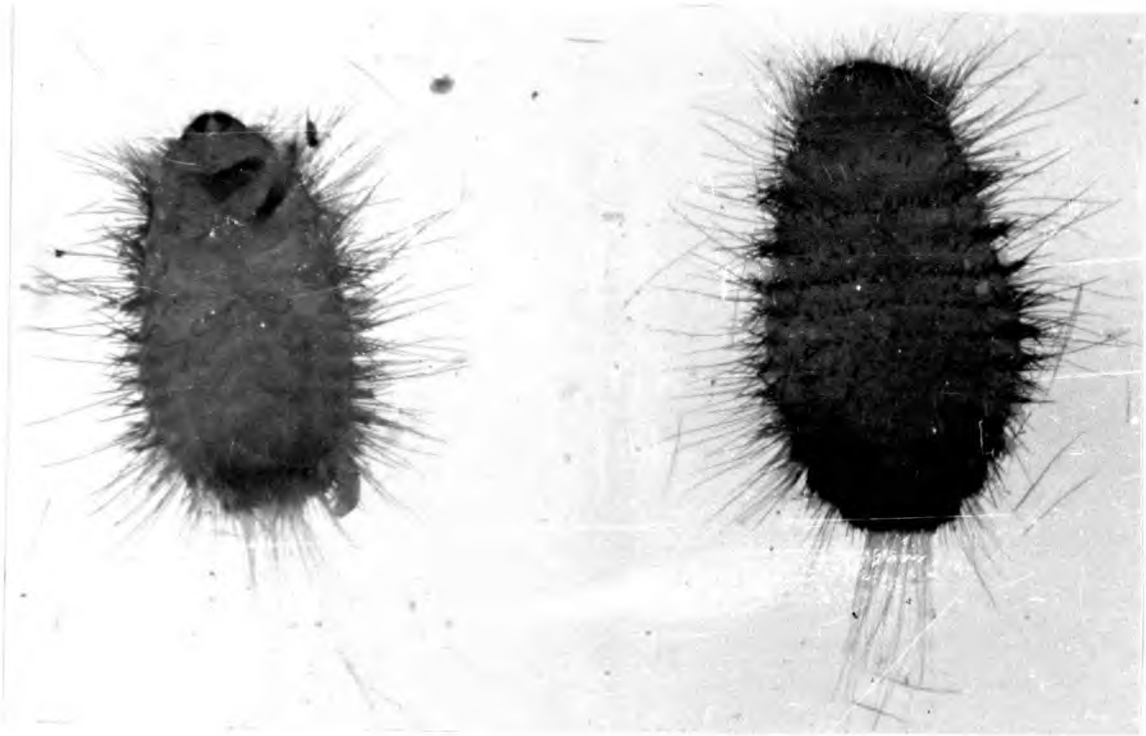
20 u.

Fig.25: The corpus cardiacum(Cc) and corpus allatum(Ca) in larva after four weeks in dormancy. Kept at 30 C and without food.



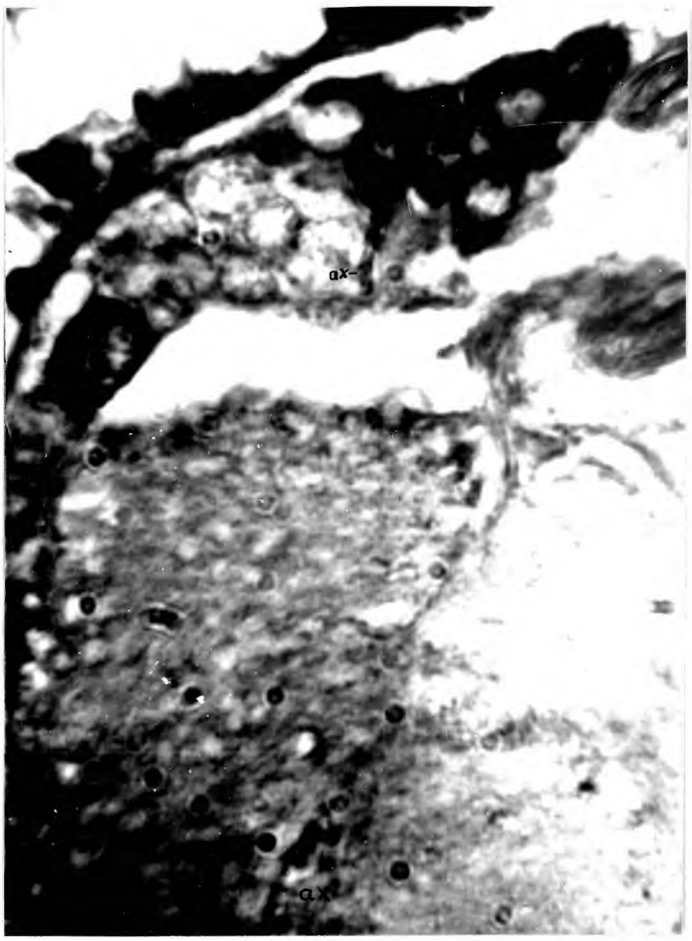
1 mm.

Fig.26. A larva, four weeks after cautery of the brain neurosecretory cells.



2 mm.

Fig.27: Larvae, three weeks after cautery of the suboesophageal ganglion neurosecretory cells.

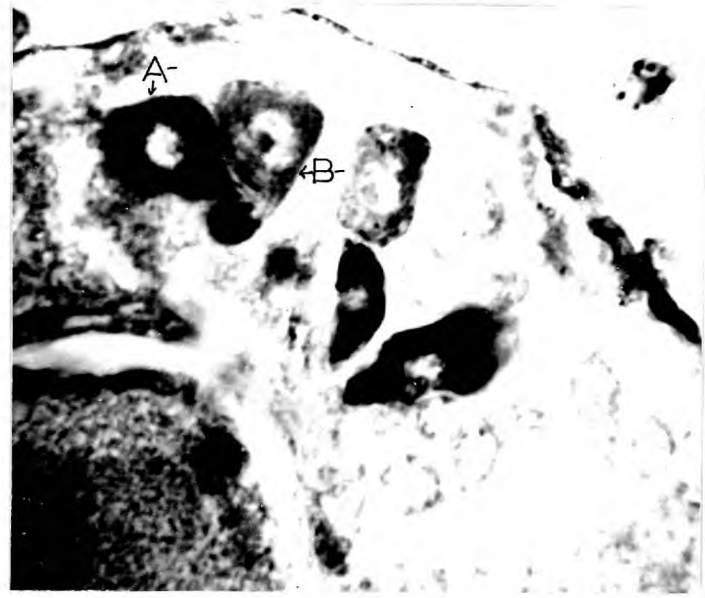


10 u.

Fig.28: The neurosecretory cells in the brain of a larva after farnesol treatment.
ax : Axons

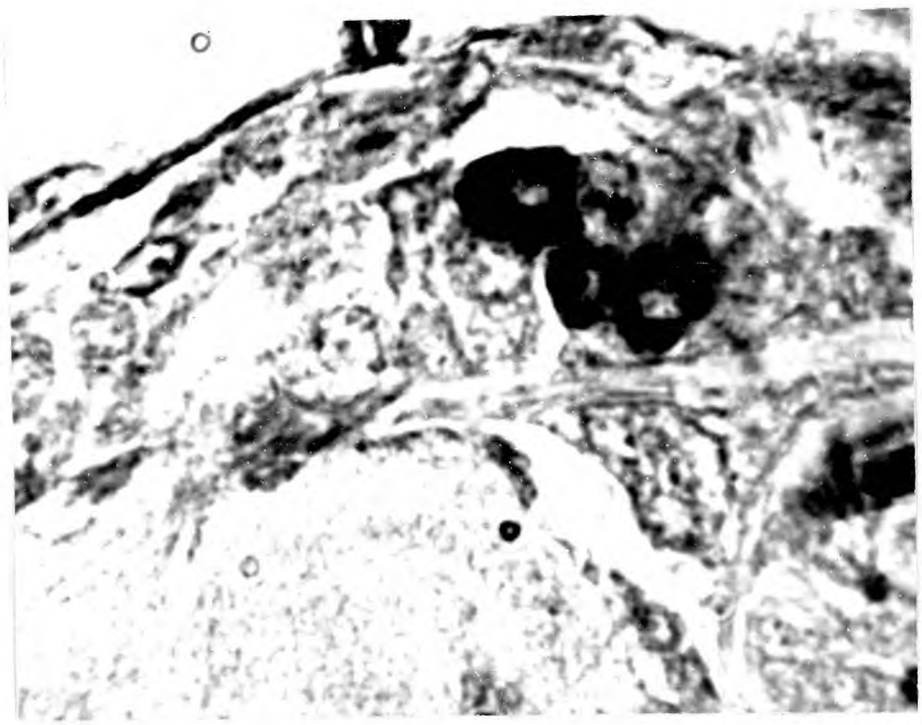
20 u.

Fig.29: The corpus allatum and cardiacum in mature larva after farnesol treatment. (Compare with fig.15); notice the accumulation of the neurosecretory material in the corpus cardiacum, and the ill-defined cell walls of the glands.



13 u.

Fig.30: A medial group of neurosecretory cells in the brain of an 0-24 hour old pupa.



12 u.

Fig.31: A lateral group of neurosecretory cells in the brain of an 0-24 hour old pupa.

Fig. 32

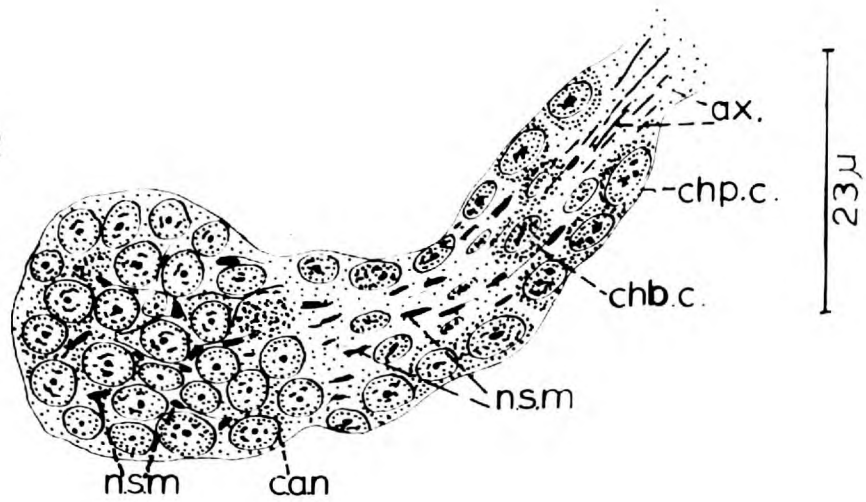
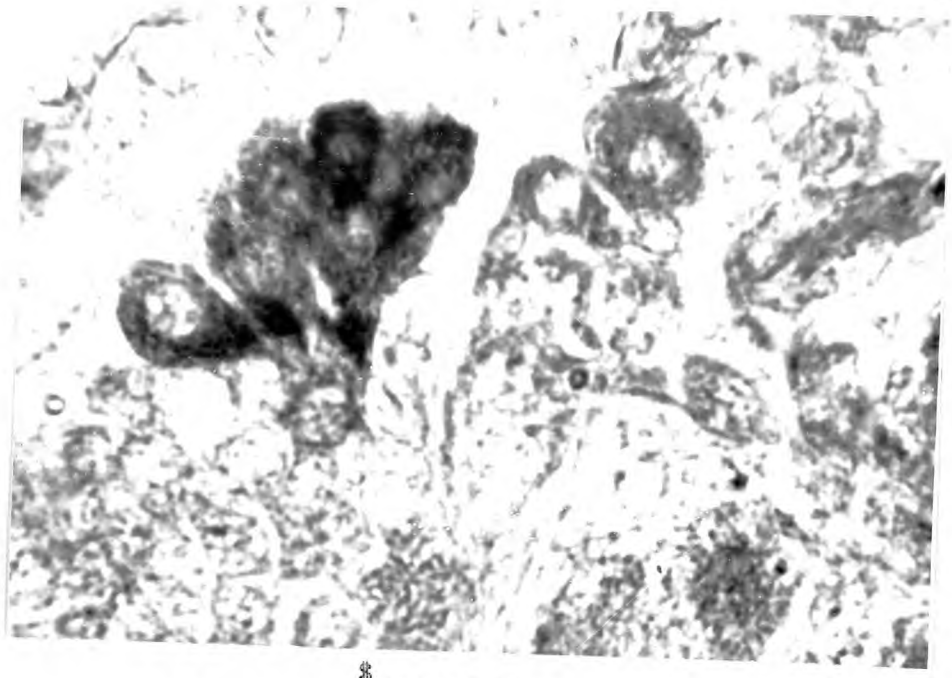


Fig.32: Corpus cardiacum and allatum in an 0-24 hour old pupa.

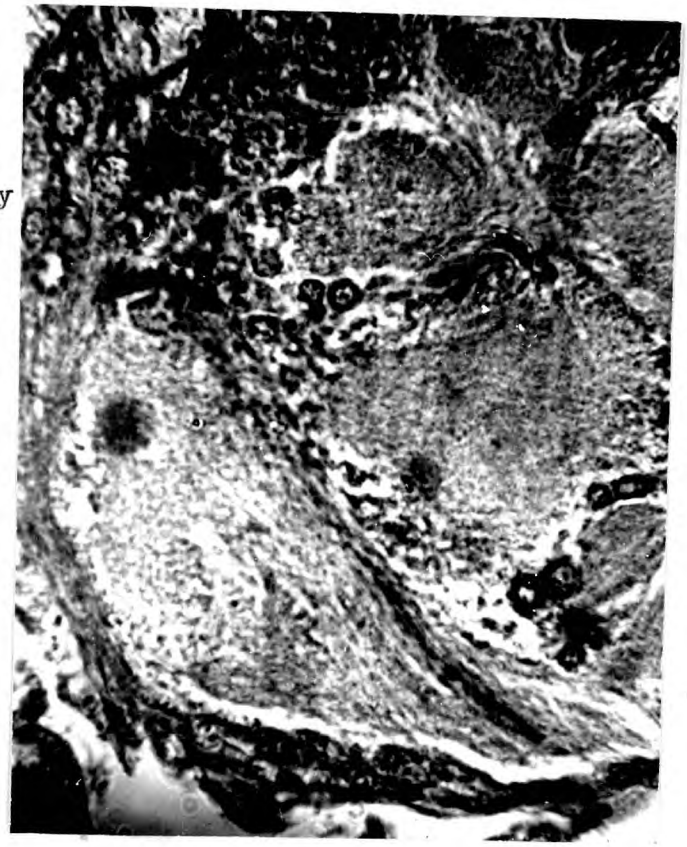
ax: axons;
c.a.n: corpus allatum nuclei;
chb.c: chromophobe cells;
chp.c: chromophile cells;
n.s.m: neurosecretory material.



16 u.

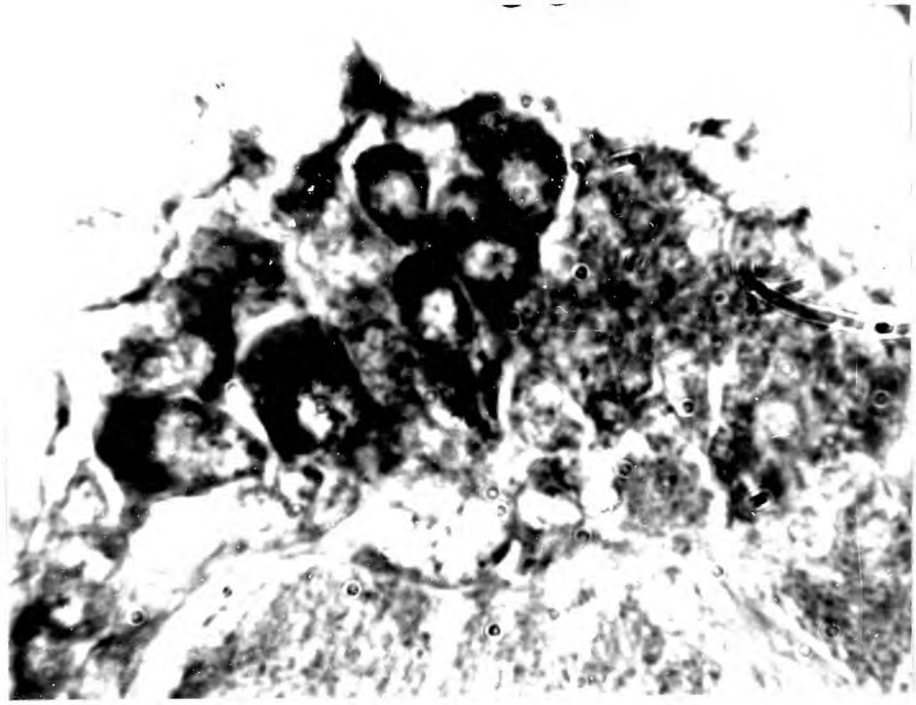
Fig. 33: Neurosecretory cells in the brain of a 24-72 hour old pupa.

Fig. 34: Axons of neurosecretory material in the brain of a 24-72 hour old pupa.



18 u.

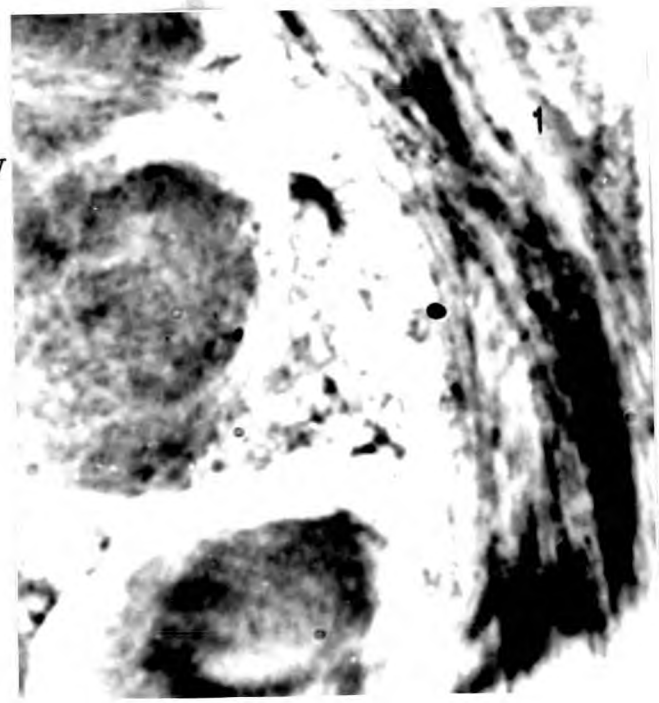
Fig.35: The corpus allatum(Ca) and cardiacum(Cc) of a 24-72 hour old pupa.



30 u.

Fig. 36: Neurosecretory cells in the brain of a 72-96 hour old pupa.

Fig:37: Axons of neurosecretory material in the brain of a 72-96 hour old pupa.



18 u.

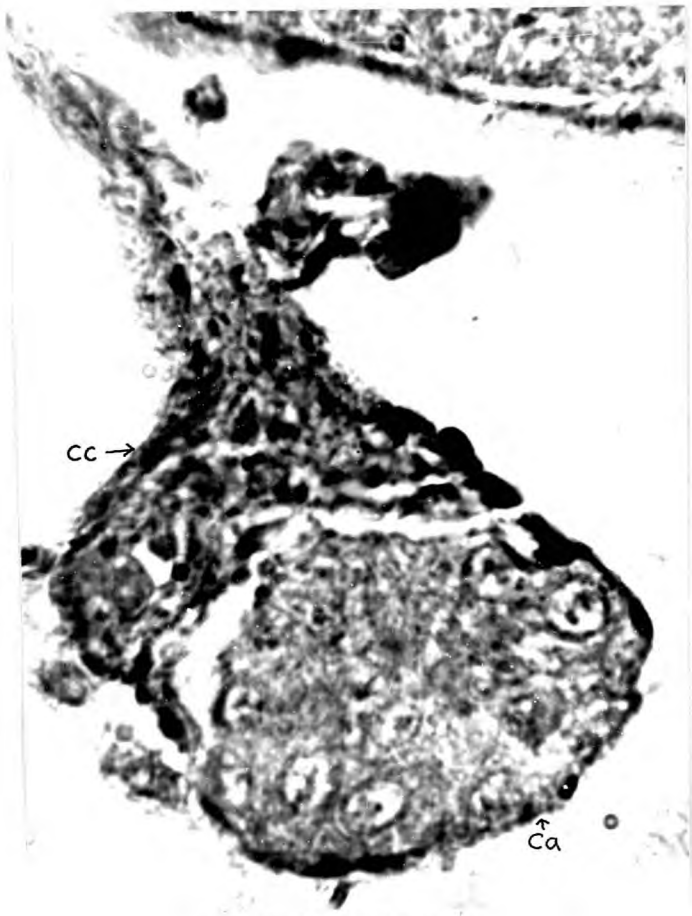


Fig. 38: Corpus allatum(Ca) and C. cardiacum(Cc) in a 72-96 hour old pupa.

Fig. 39: A-cell in the suboesophageal ganglion of a 72-96 hour old pupa.

A

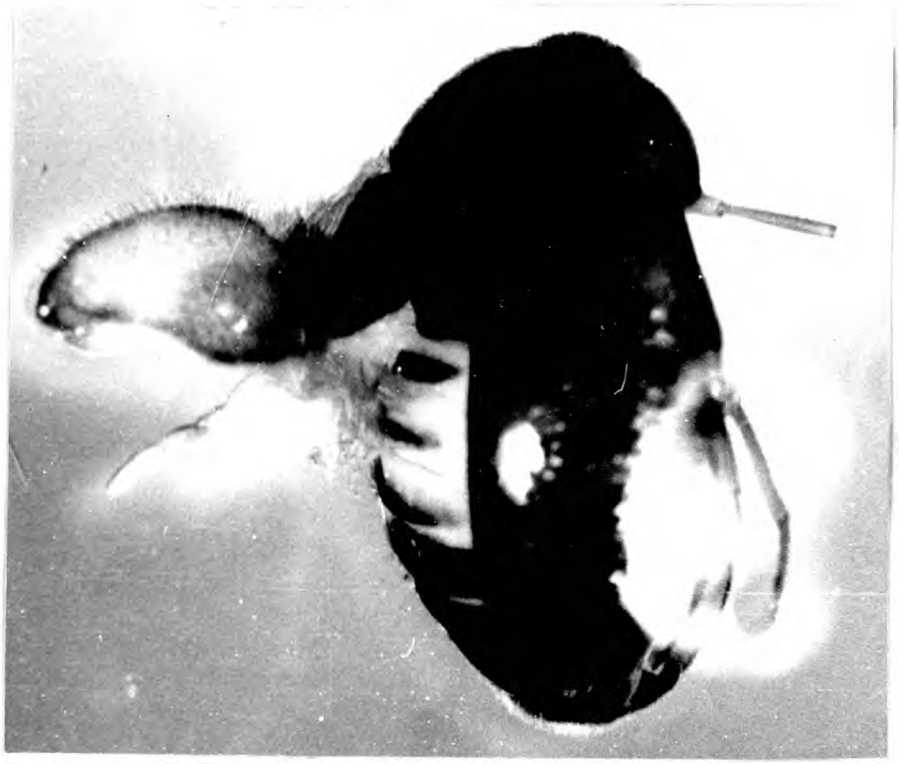
1mm.

B

1mm.

Fig.40: T. granarium after smearing 24-72 hour old pupa with farnesol.

A :Pupa with little differentia tion ;
B :Adult with pupal setae.



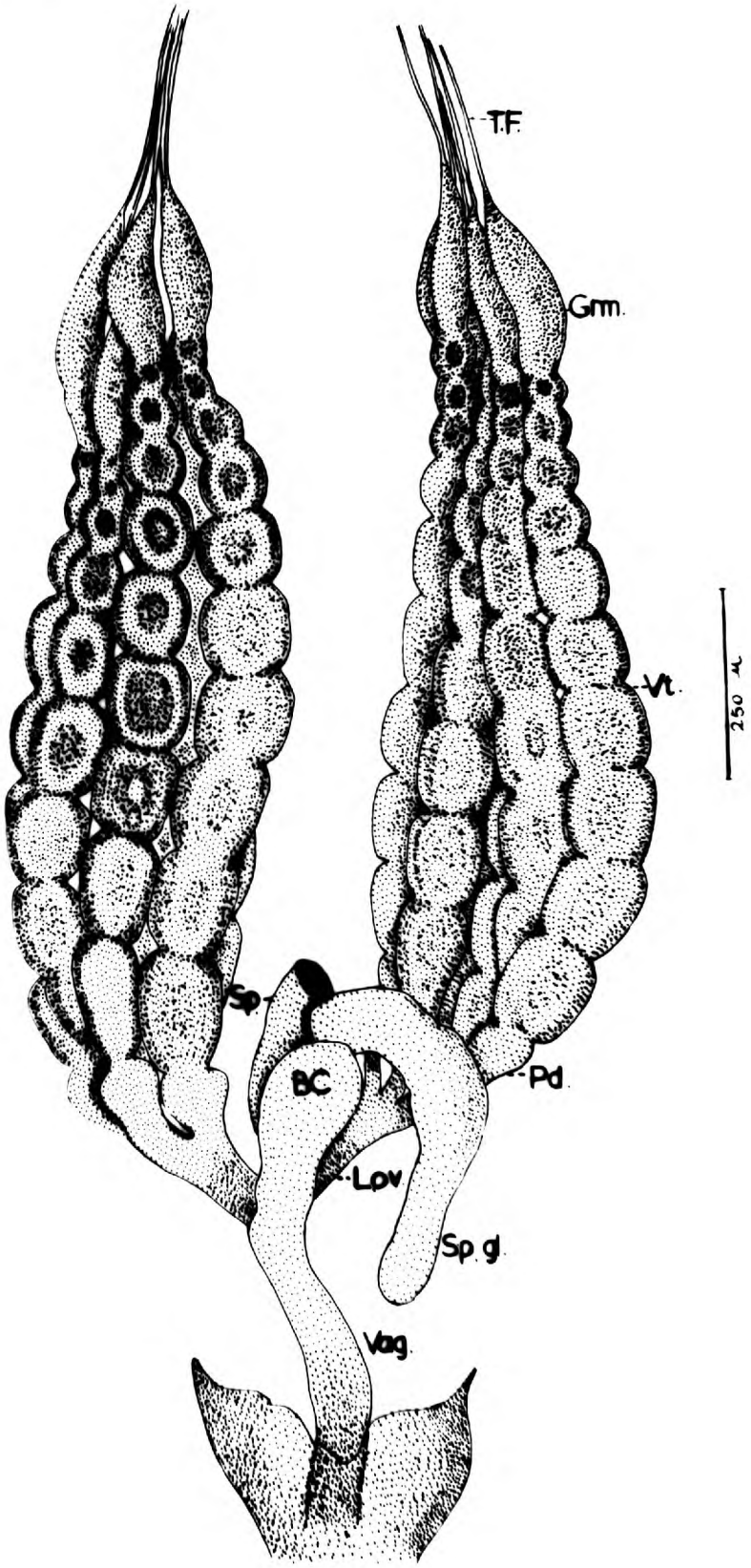
1 mm.

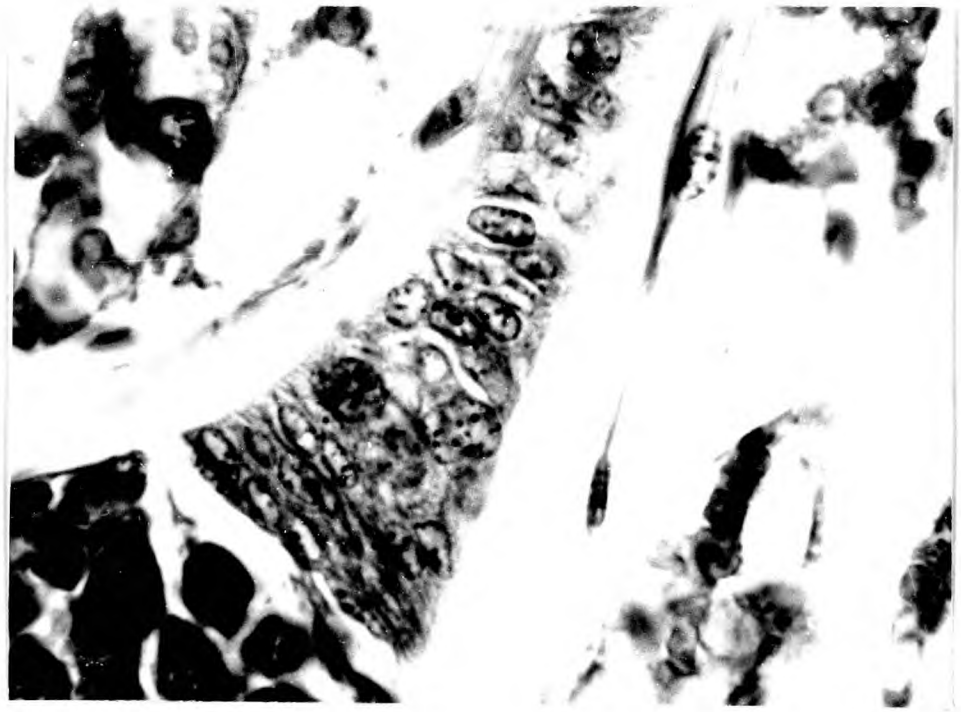
Fig. 41: A deformed adult, after smearing old pupa with farnesol.

Fig. 42: Female reproductive system.

BC: bursa copulatrix;
Grm: germarium;
L.ov: lateral oviduct;
Pd: pedicel;
Sp: spermatheca;
Sp.gl: spermathecal gland;
TF: terminal filaments;
Vag: vagina;
Vt: vitelleria.

Fig. 42.





30 u.

Fig. 43A: L.S. Terminal filament. For details see figure (43b)

Fig. 43b

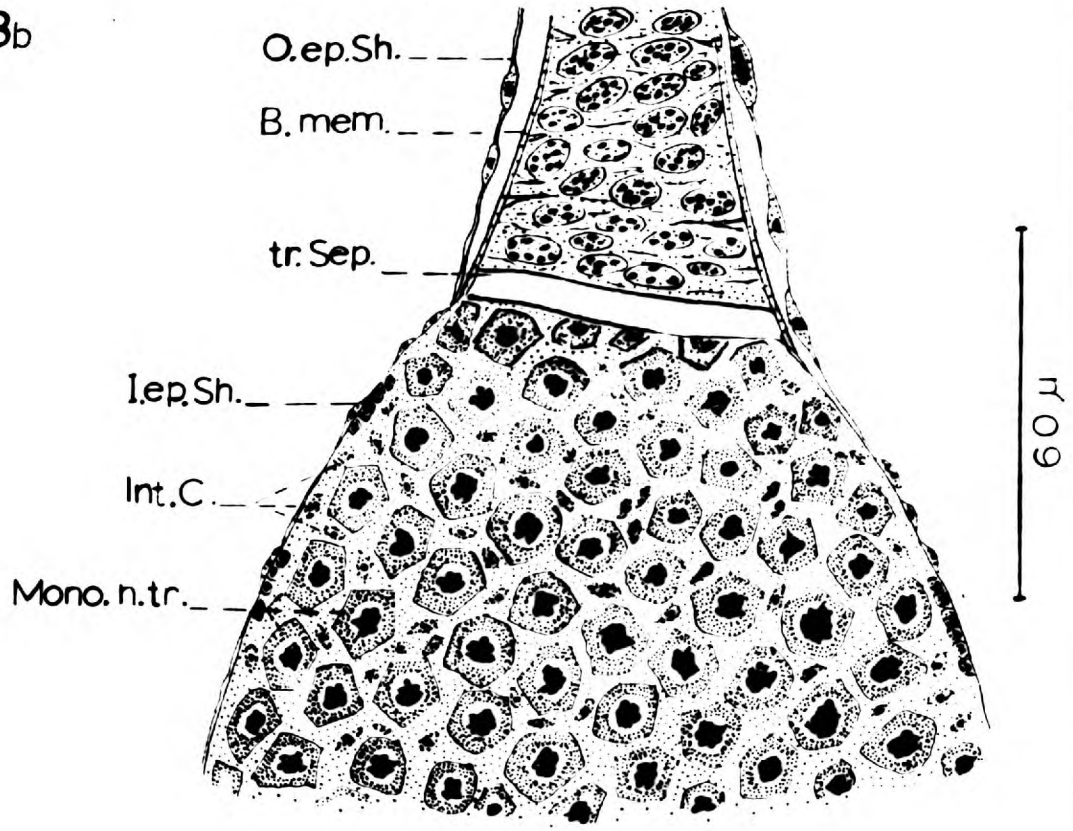


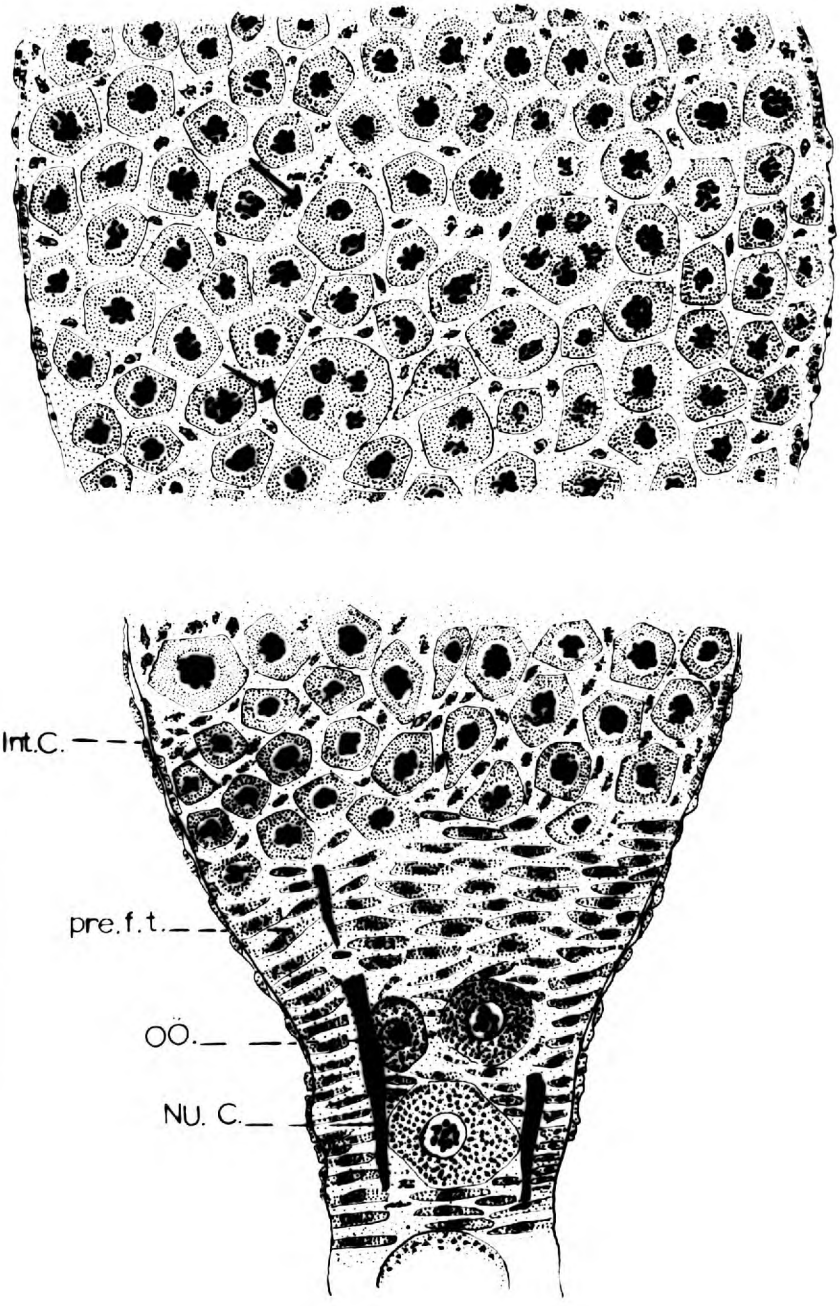
Fig. 43: The distal region of the germarium.

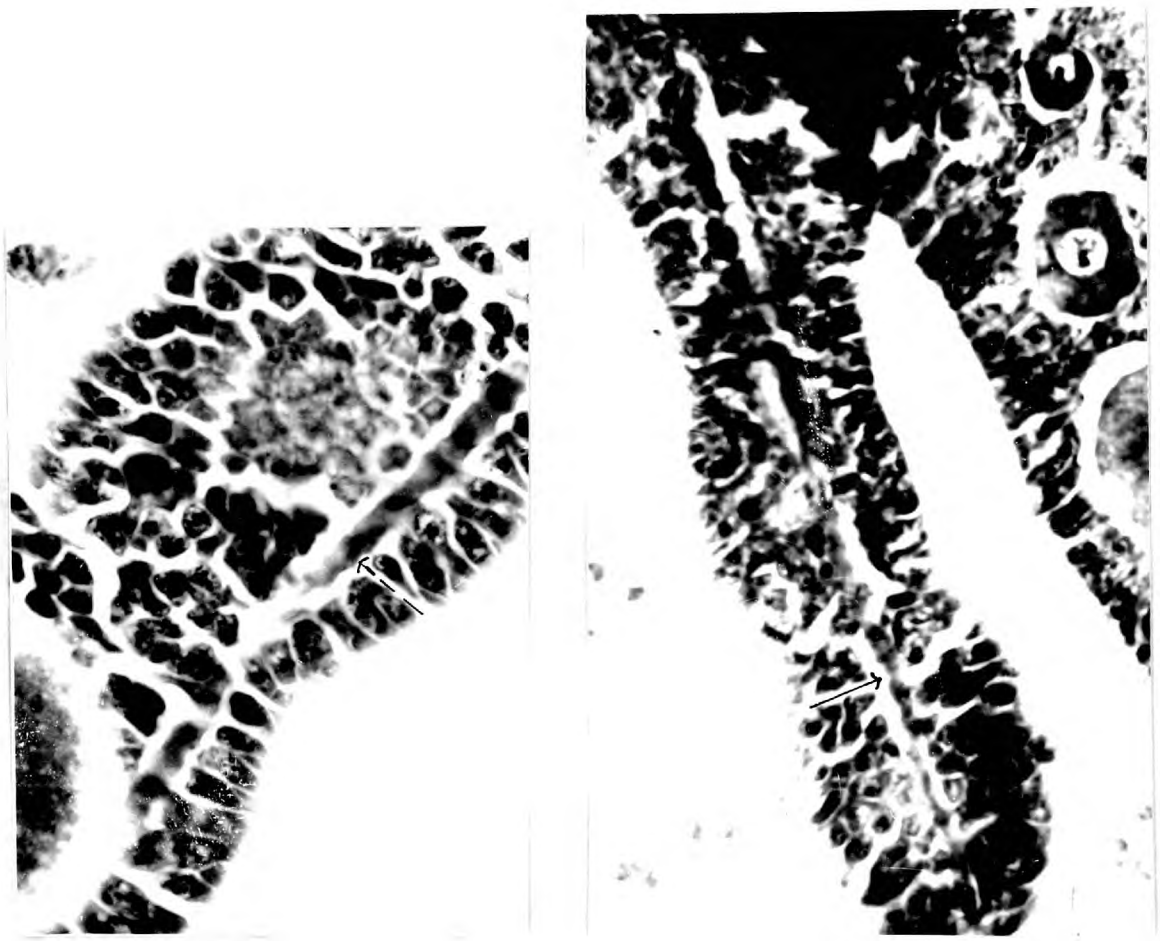
B.mem: basement membrane;
I.ep.sh: inner epithelial sheath;
Int.C: interstitial cells;
Mono.n.tr: mononucleate trophocytes;
O.ep.sh: outer epithelial sheath;
tr.sep: transverse septum.

Fig. 44: The middle and posterior regions of the germarium.

Bi.n.tr: binucleate trophocytes;
Int.C: interstitial cells;
Multi.n.tr: multinucleate trophocytes;
NU.C: nutritive cord;
OO: oocyte;
pre.f.t: prefollicular tissue.

Fig. 44.



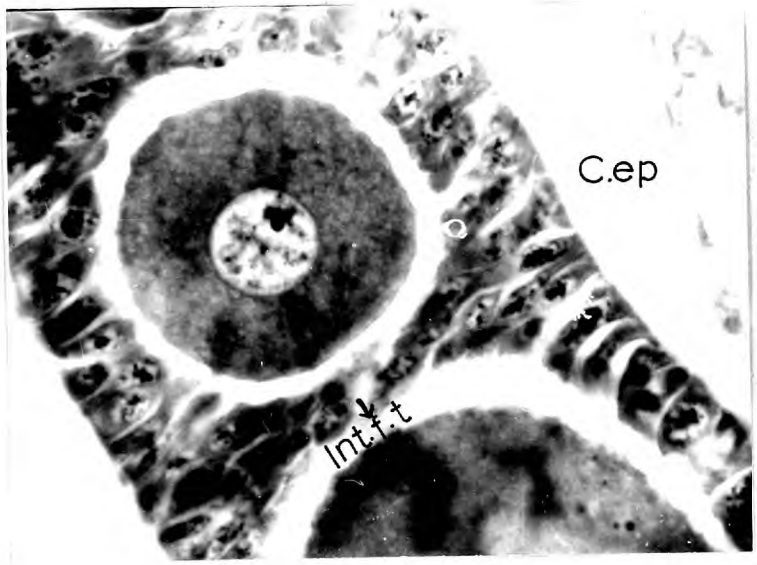


(45) (46)

20 u.

The nutritive cord.

Fig. 45: showing the nutritive cord passing from one oocyte to another;
Fig. 46: showing the nutritive cord passing from one follicle to another.



18 u.

Fig.47: L.S. through the ovary showing an oocyte with columnar epithelium.
C.ep: columnar epithelium;
Int.f.t: interfollicular tissue.

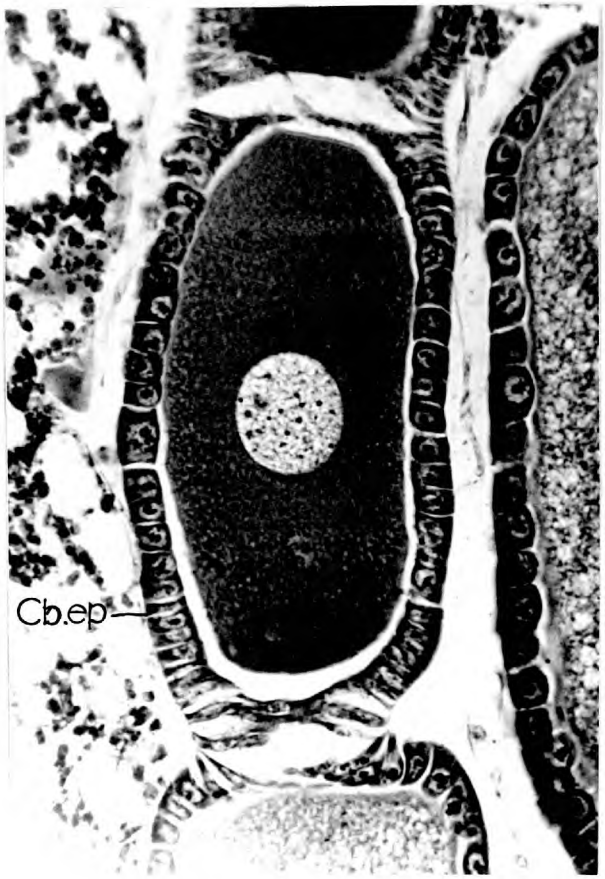
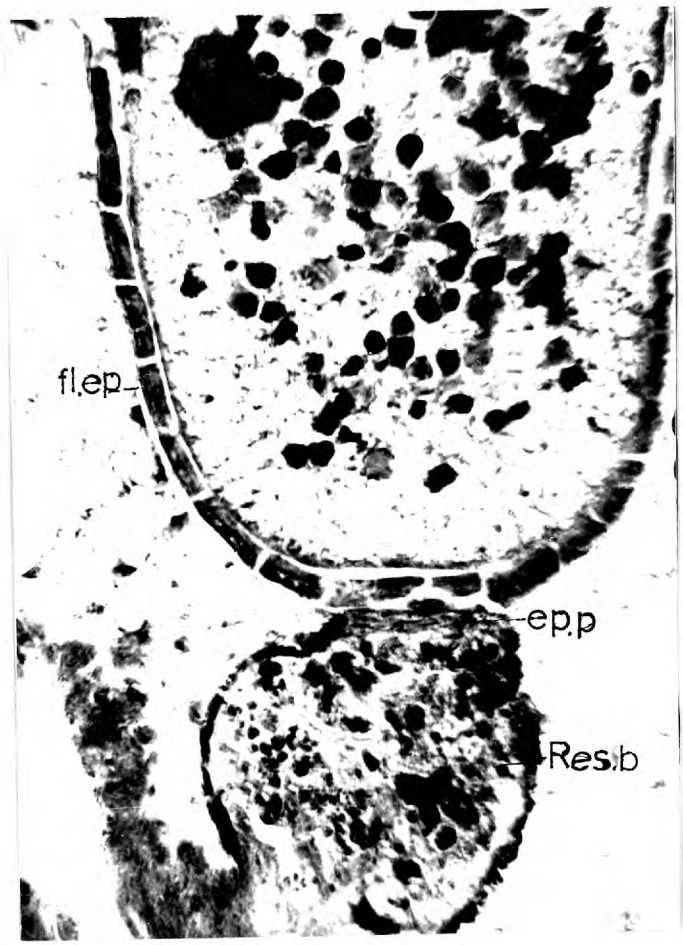


Fig.48: L.S. through the ovary showing an oocyte with cuboidal epithelium.
Cb.ep: cuboidal epithelium.

56 u.



40 u.

Fig. 49: The resorption body; (Res.b.)

ep.p: epithelium plug;
fl.ep: flattened epithelium.

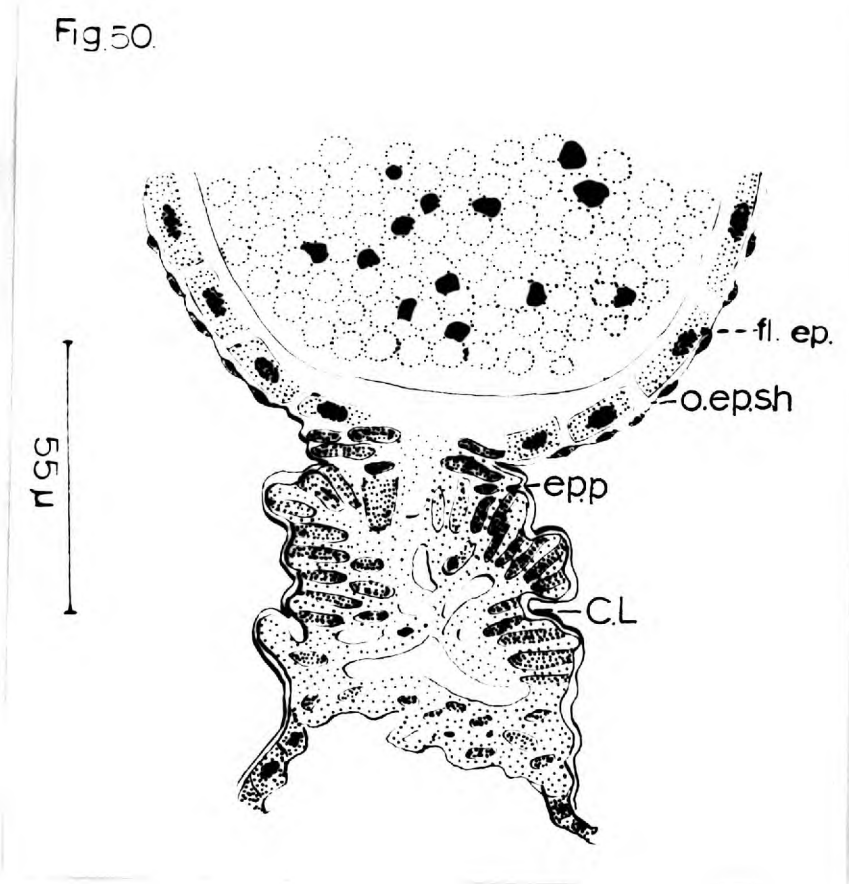
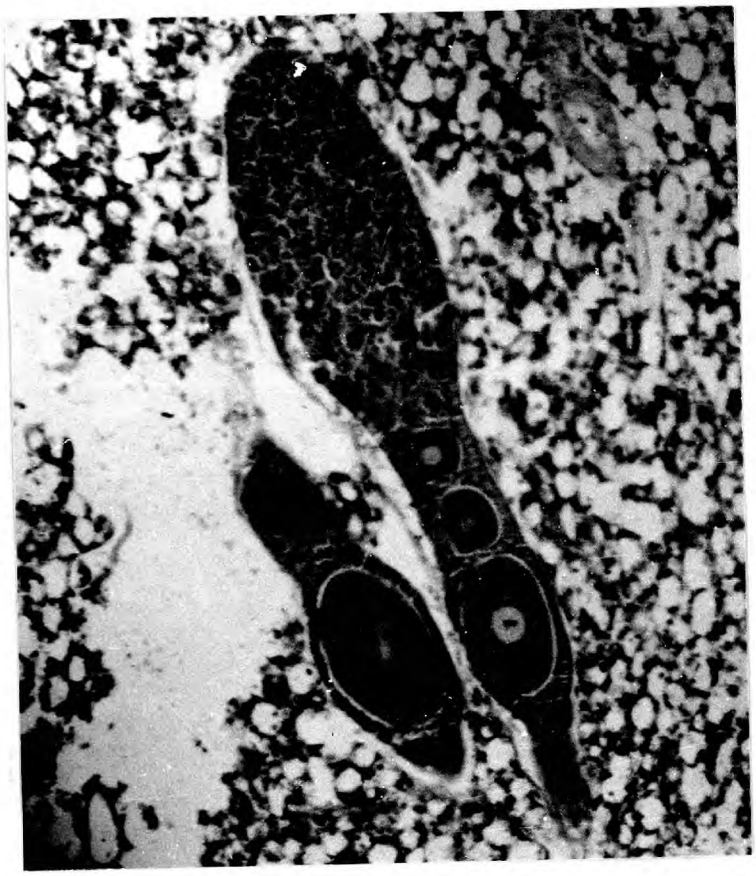


Fig.50: The corpus luteum; (C.L.)

ep.p: epithelial plug;
fl.ep: flattened epithelium;
o.ep.sh: outer epithelial sheath.



60 u.

Fig.51: An ovariole of an 0-3 hour old adult.

Fig.52: An ovary in unmated female with collapsed terminal oocytes.

95 u.

Fig. 53: An ovariole of an old adult just after the post-oviposition period.

Fig.54: The formation of resorption body.

dis.vm; disintegrated vitelline membrane;
E.F.c: invaded follicle cells;
F.c: follicle cells;
Lec.c: lecitholytic cells;
o.e.s: outer epithelial sheath;
va: vacuoles;
V.F.c: vacuolated follicle cells;
v.m: vitelline membrane;
w.v.m: wrinkled vitelline membrane;
yk: yolk;
yk.g: yolk granules.

Fig. 54.

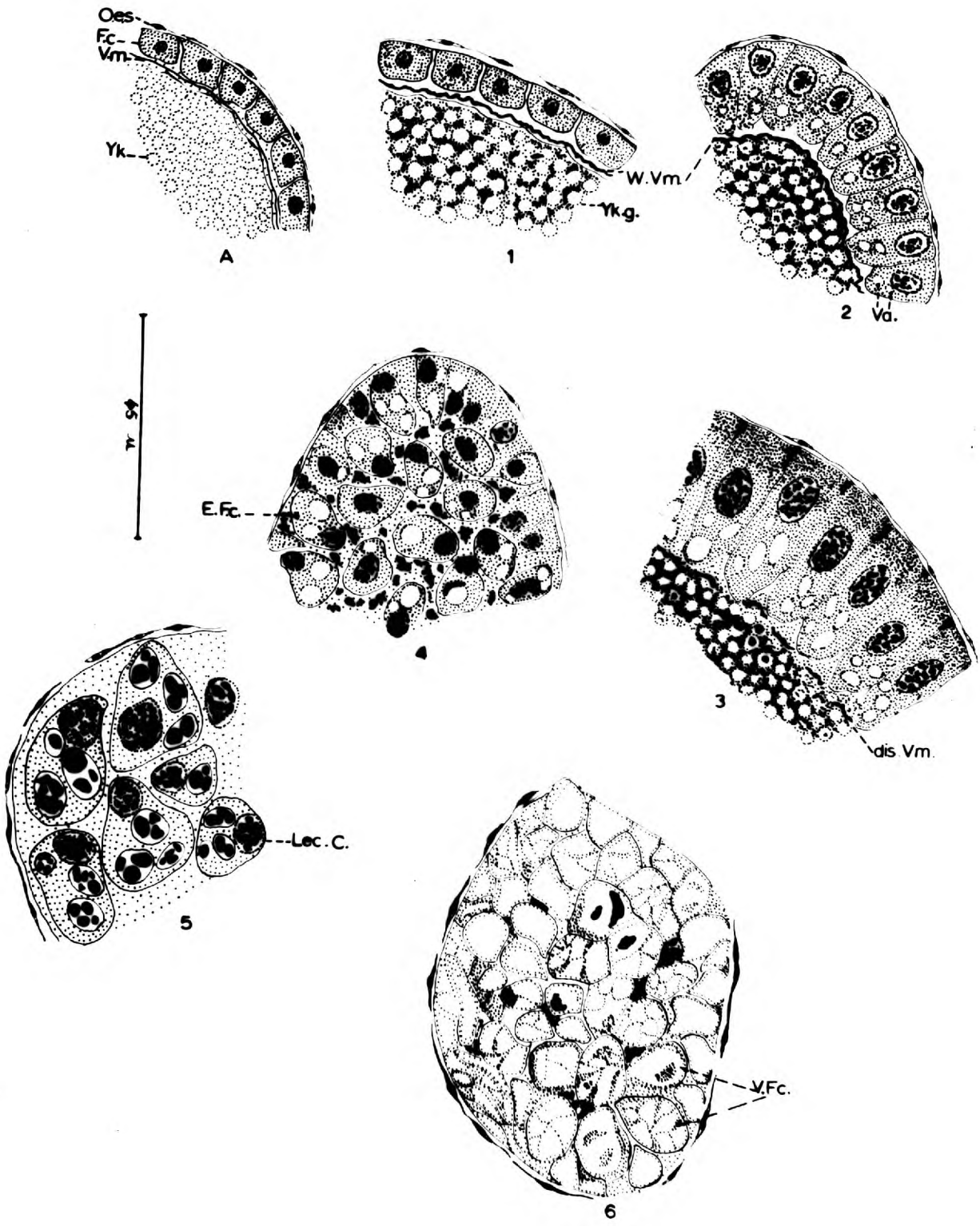


Fig.55: Male reproductive system.

Acc.gl: accessory glands;
D.ej: ductus ejaculatoris;
l.Abd.g: last abdominal ganglion;
Ts: testes;
V.d:vasa deferentia.

Fig 55

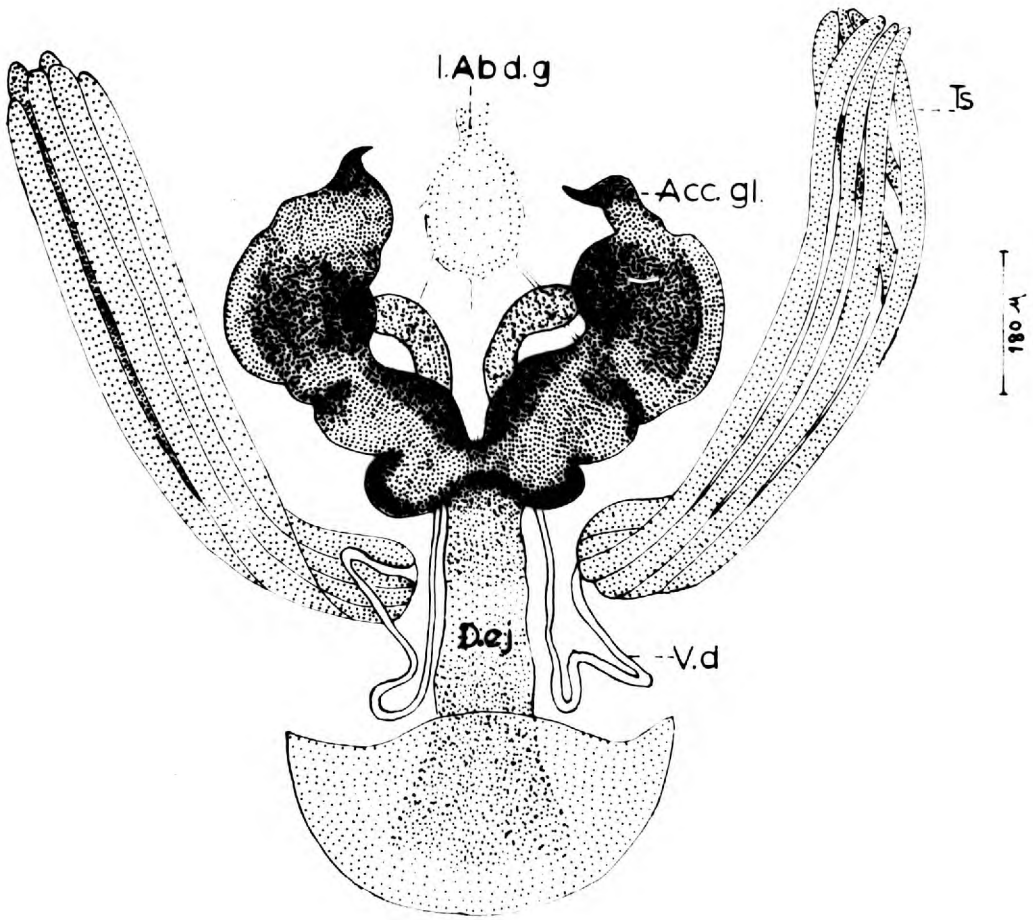
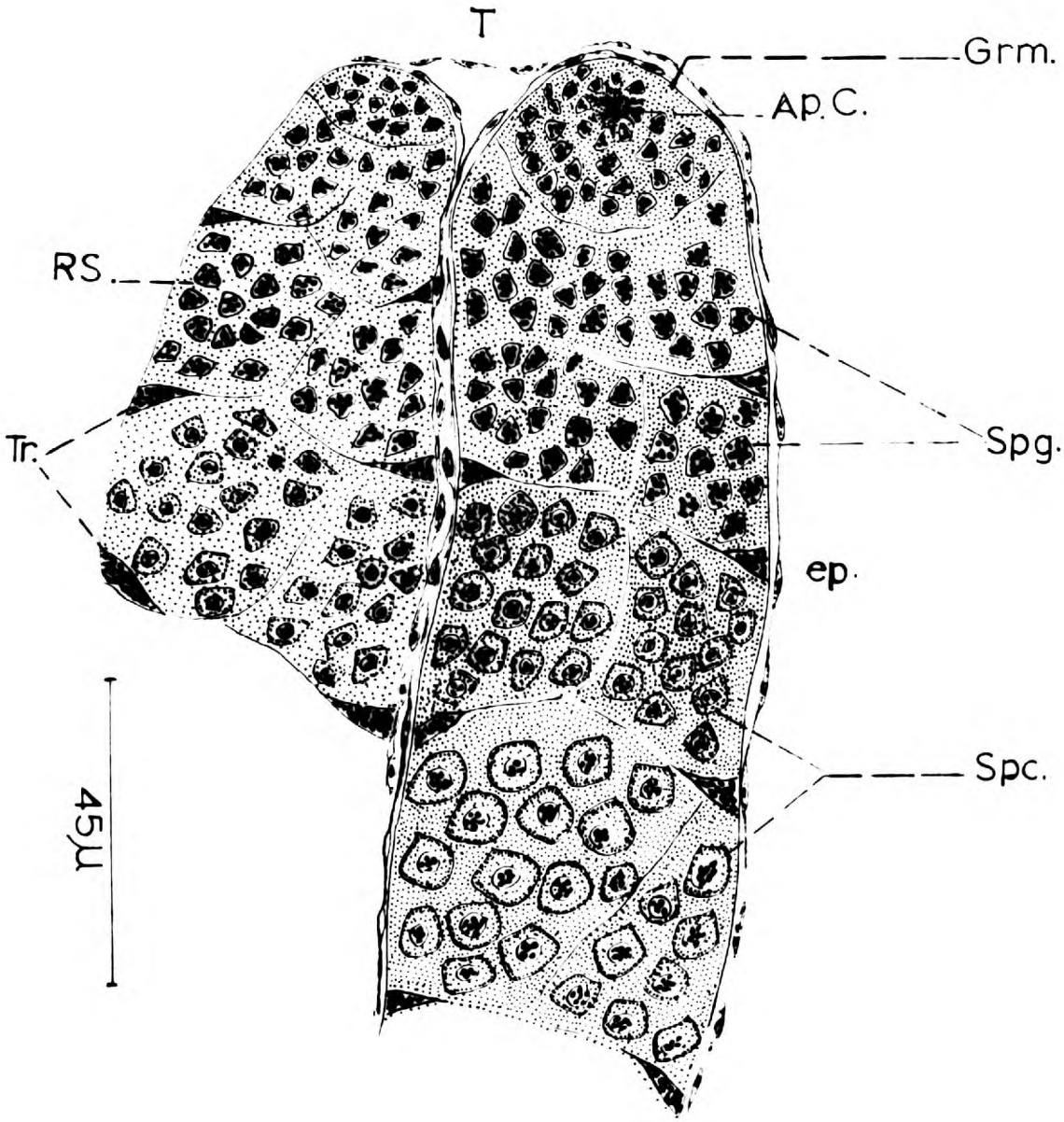
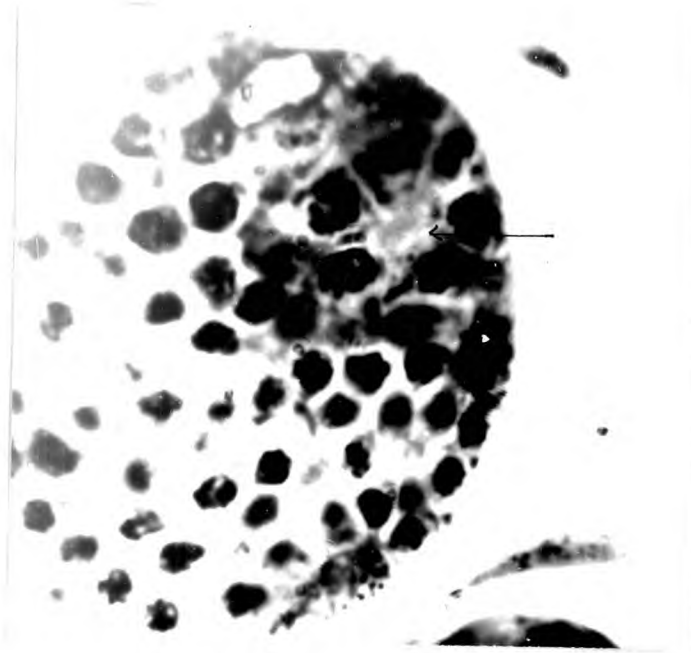


Fig.56: L.S. through testis follicles.

Ap.c: apical cell;
ep: epithelial sheath;
Grm: germarium;
RS: rosette;
Spc: spermatocytes;
Spg: spermatogonia;
T: tunica;
Tr. trophocytes.

Fig. 56.





14 u.

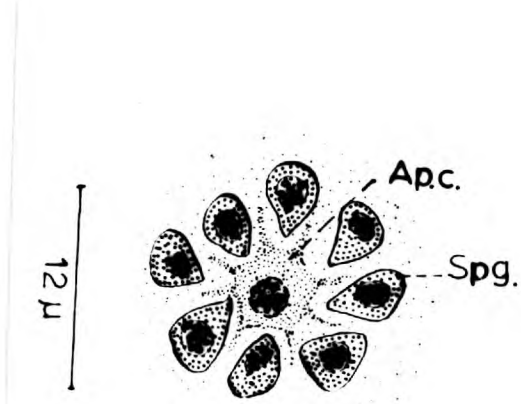
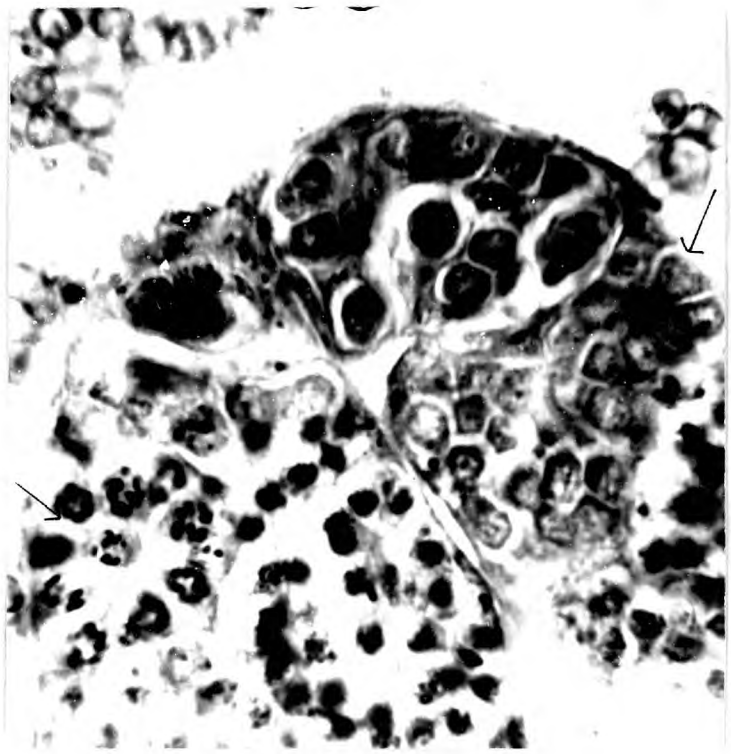


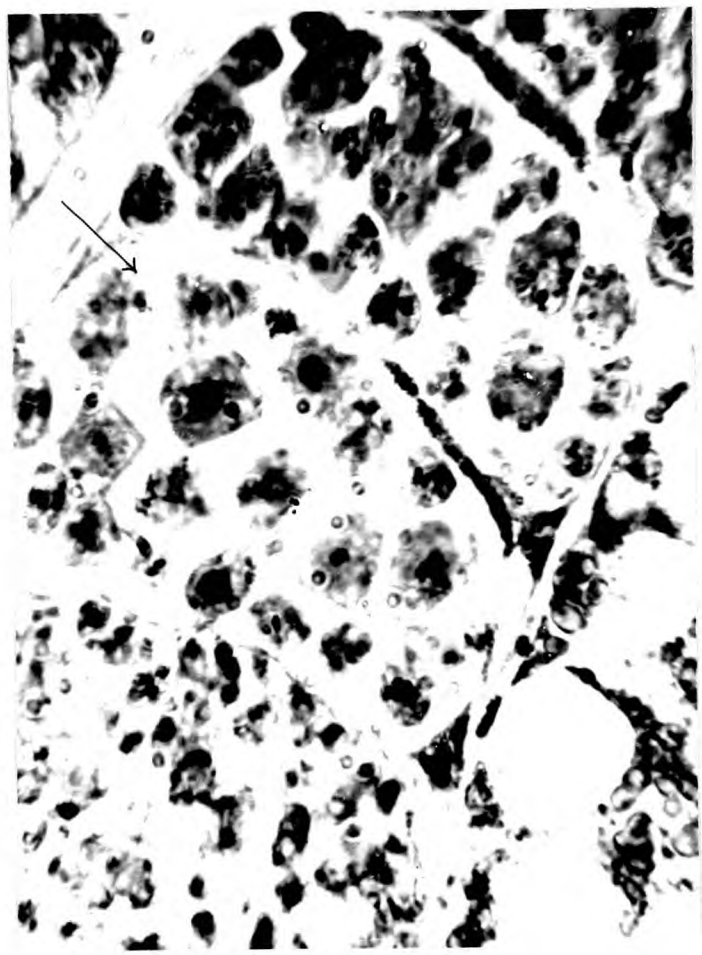
Fig.57: L.S. through the distal region of the testis showing the apical cell.

Ap.c: apical cell;
Sp.g: spermatogonia.



45 u.

Fig.58: L.S. through the testis showing the rosettes.



45.u.

Fig.59: L.S. through the testis showing the spermatocytes.

45 u.

Fig.60: L.S. through the testis showing the spermatids and spermatozoa.

spt: spermatids
spz: spermatozoa.

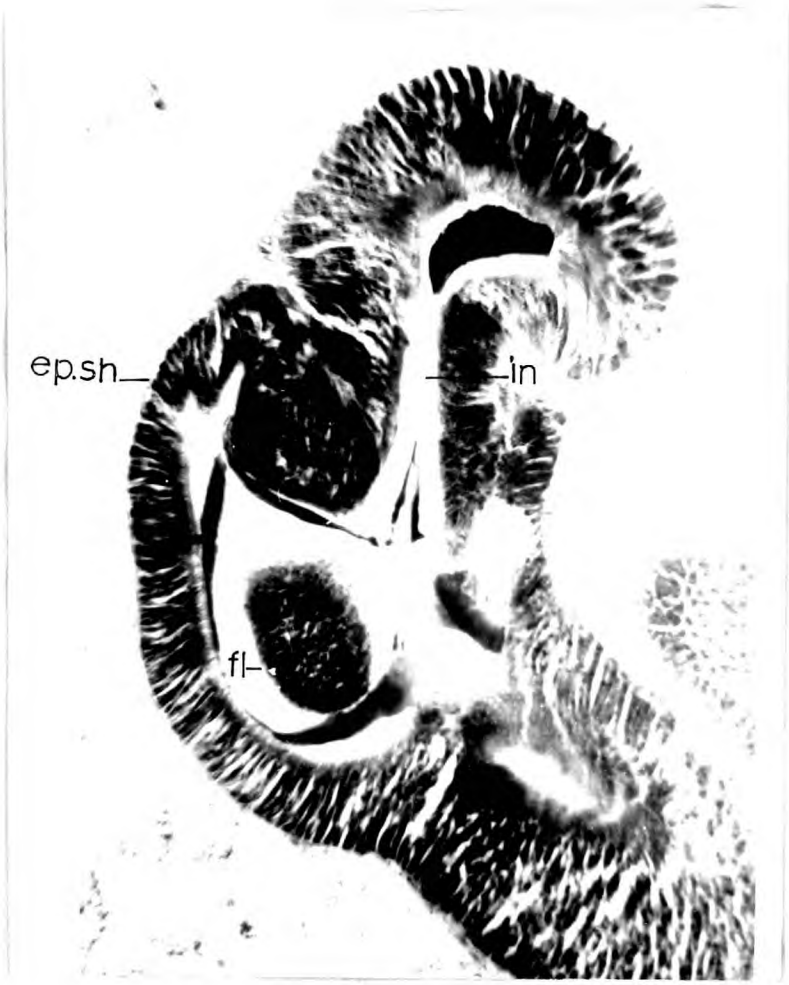


Fig.61: L.S. Accessory gland in the male.

ep.sh: epithelial sheath;
in: intima;
fl: fluid.

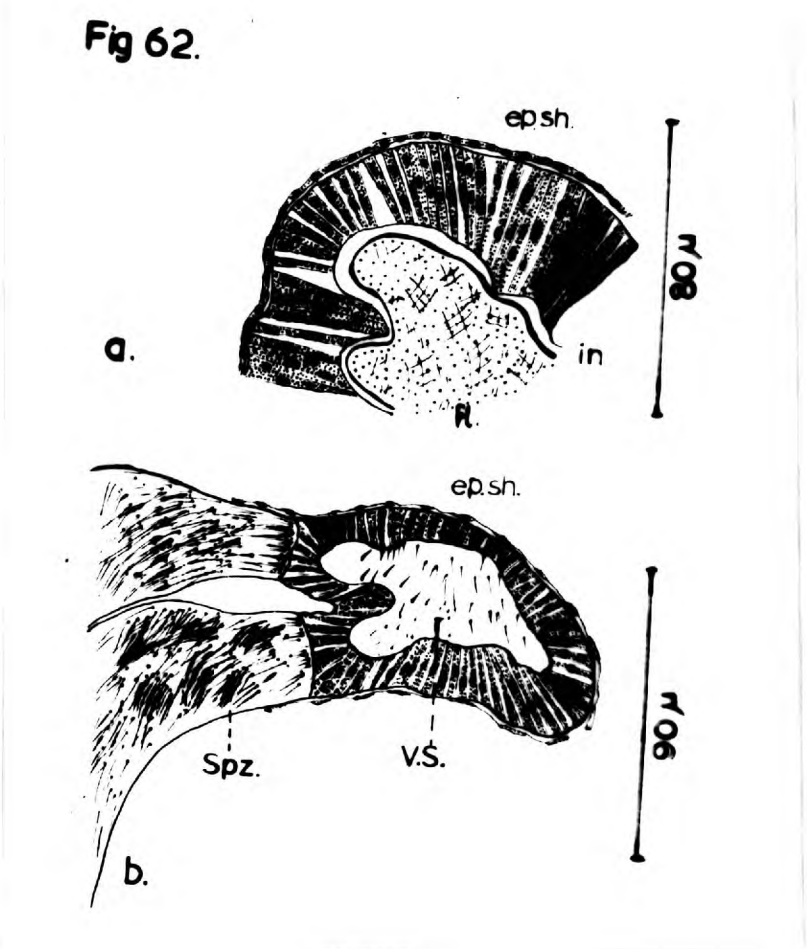


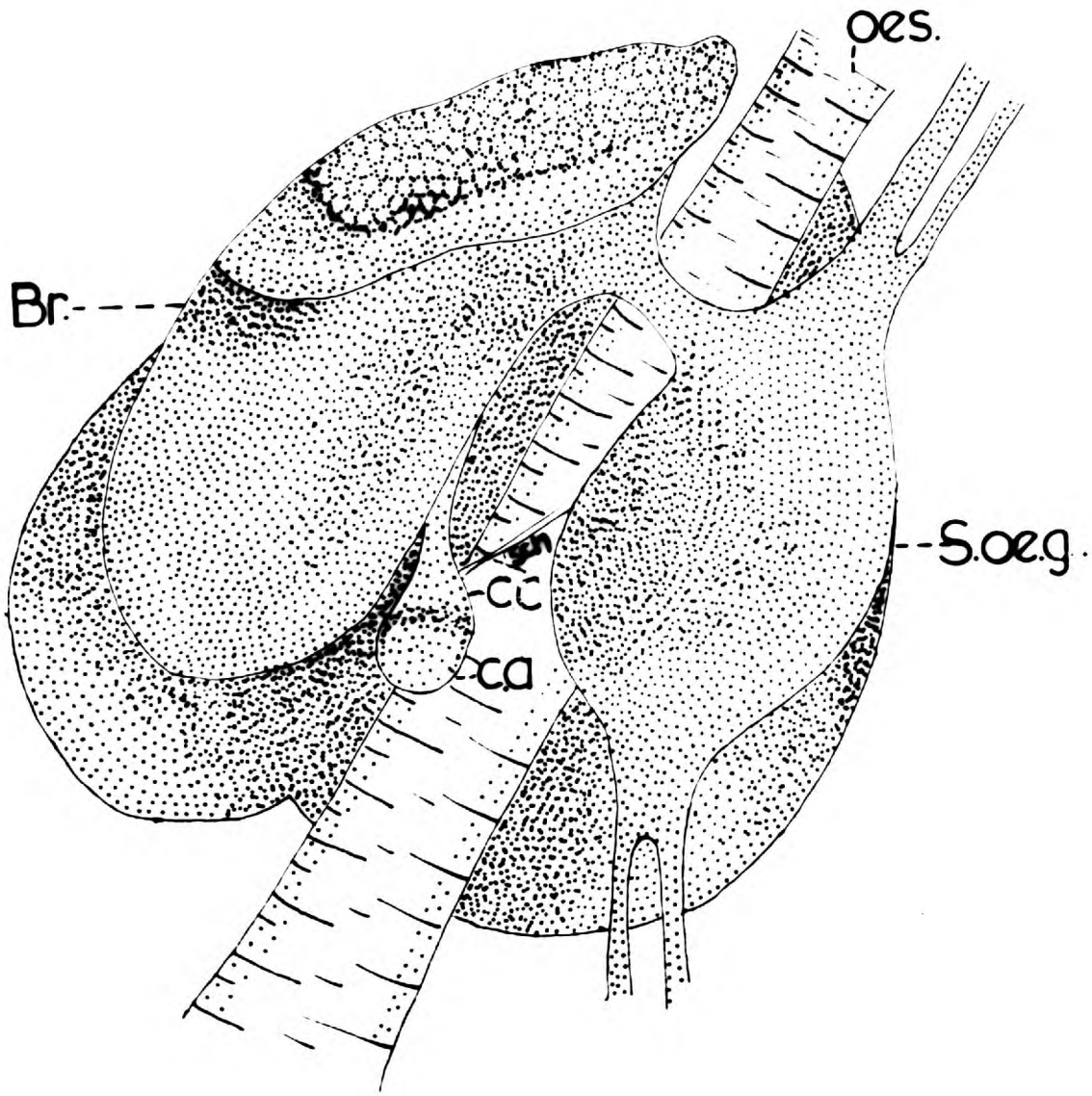
Fig.62: L.S. (a) region of accessory gland in the male;
(b) vesicula seminalis;

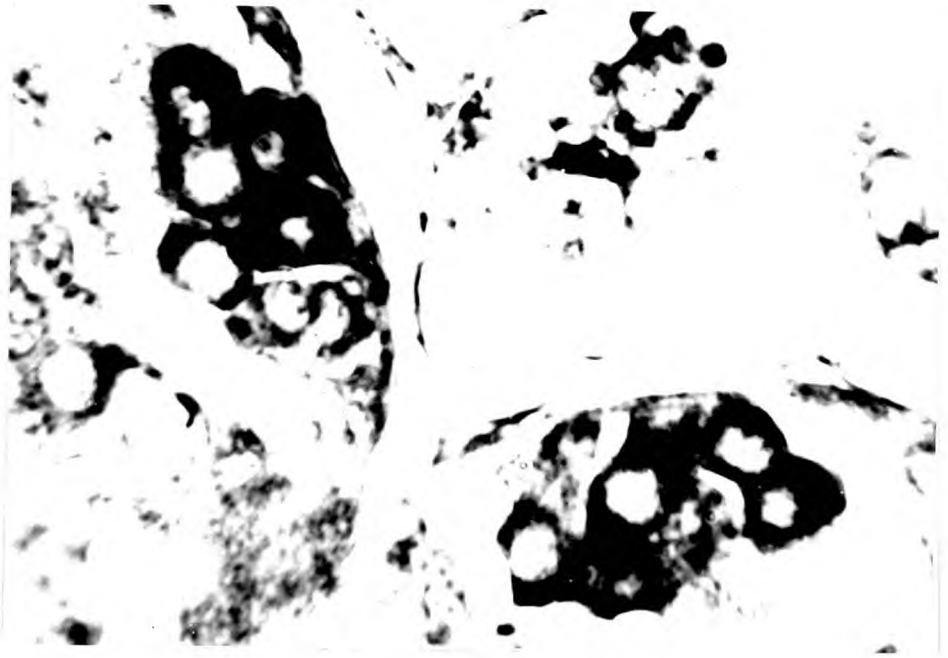
V.S: vesicula seminalis;
Spz: spermatozoa.
For other letterings see previous figure.

Fig.63: Dorso-ventral aspect of the neuro-endocrine system in the adult.

Br: brain;
Ca: corpus allatum;
Cc: corpus cardiacum;
oes: oesophagus;
S.oe.g: suboesophageal ganglion;
s.c.n: suboesophageal-corpara nerve.

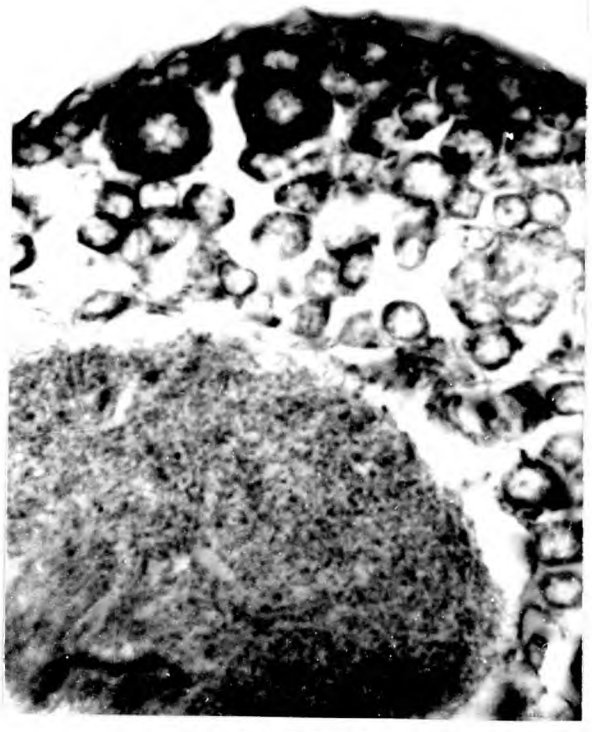
Fig 63





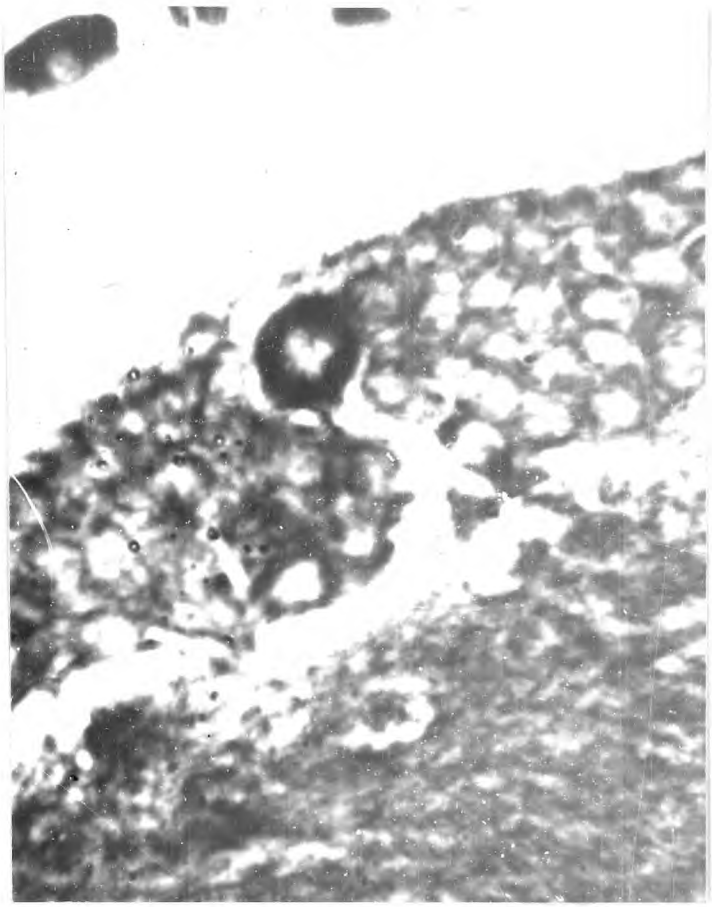
20 u.

Fig.64: The two medial groups of neurosecretory cells in the brain of mature adult.



20 u.

Fig.65: A lateral group of neurosecretory cells in the brain of mature adult.



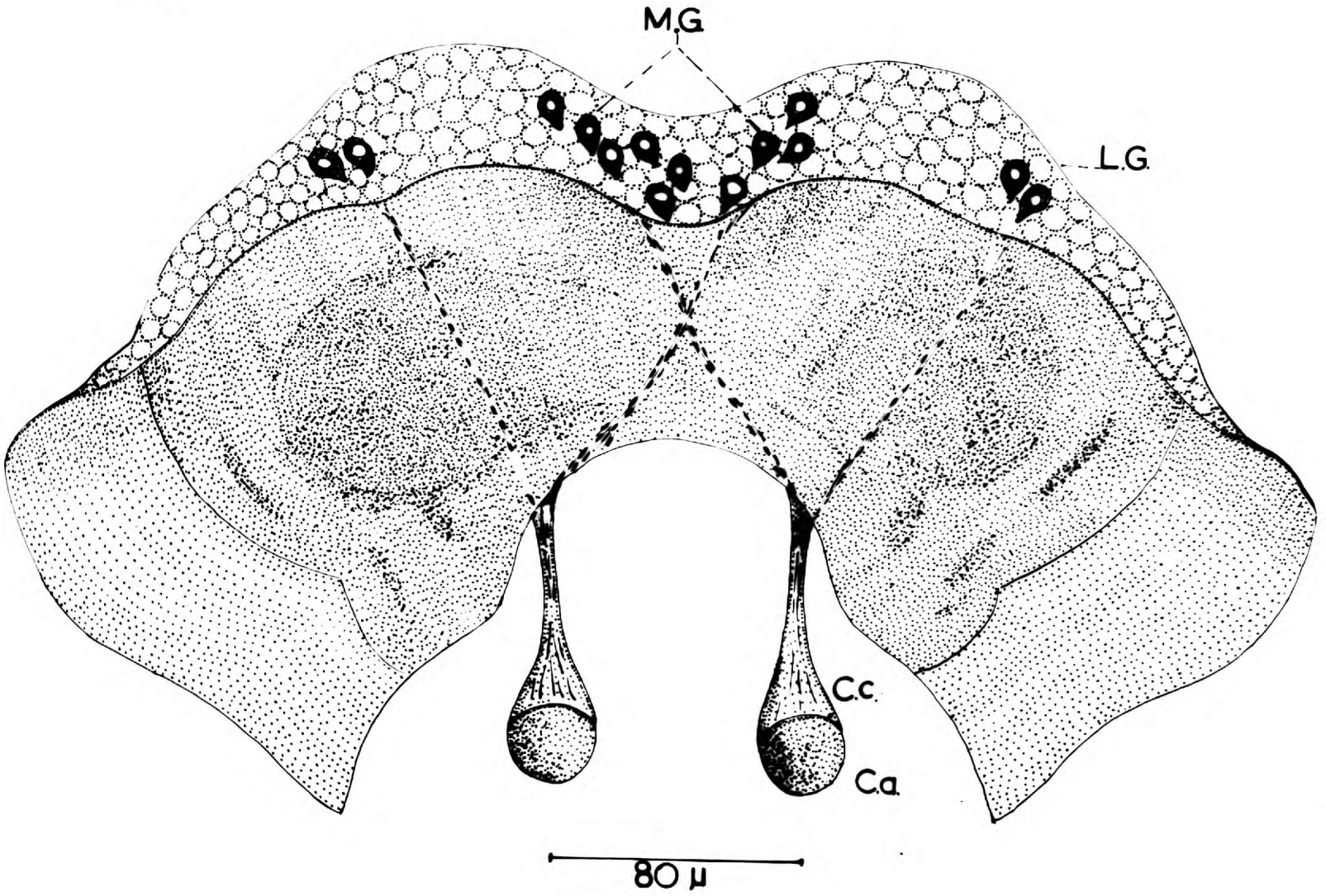
15 u.

Fig. 66: The A-cell in the suboesophageal ganglion of adult T. granarium.

Fig.67: Arrangement of Neurosecretory Cell groups and their pathways in the brain of the adult.

C.a: corpus allatum;
C.c: corpus cardiacum;
L.G: lateral group;
M.G: medial groups.

Fig. 67.



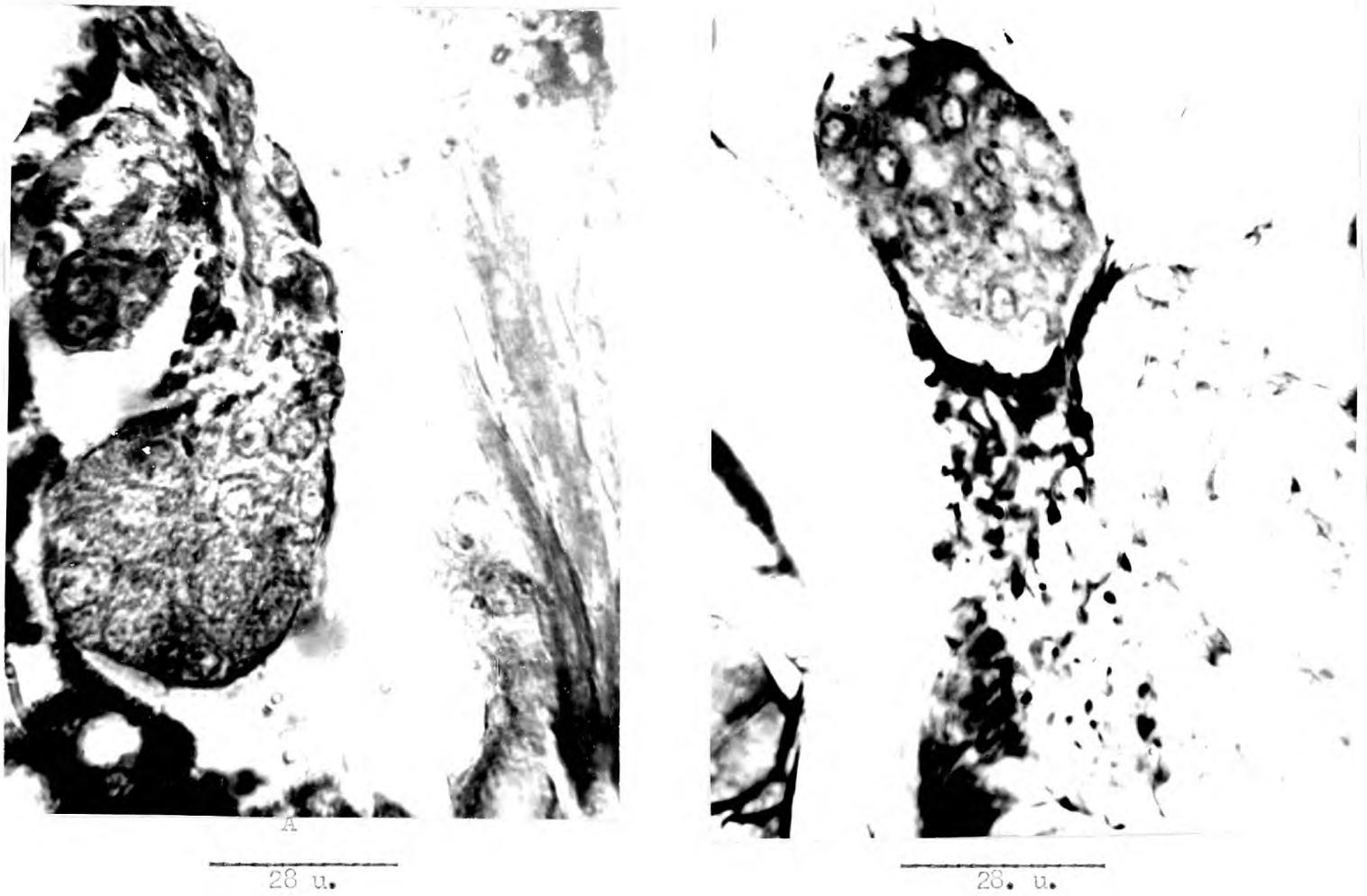
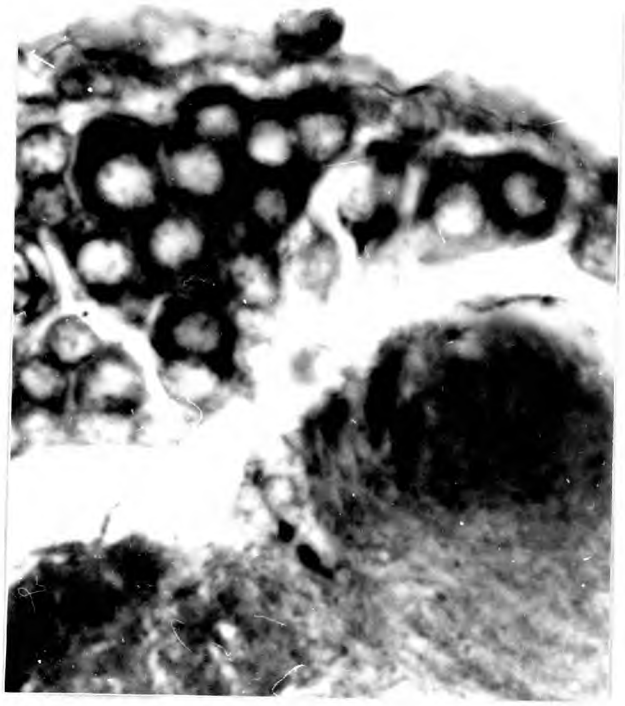


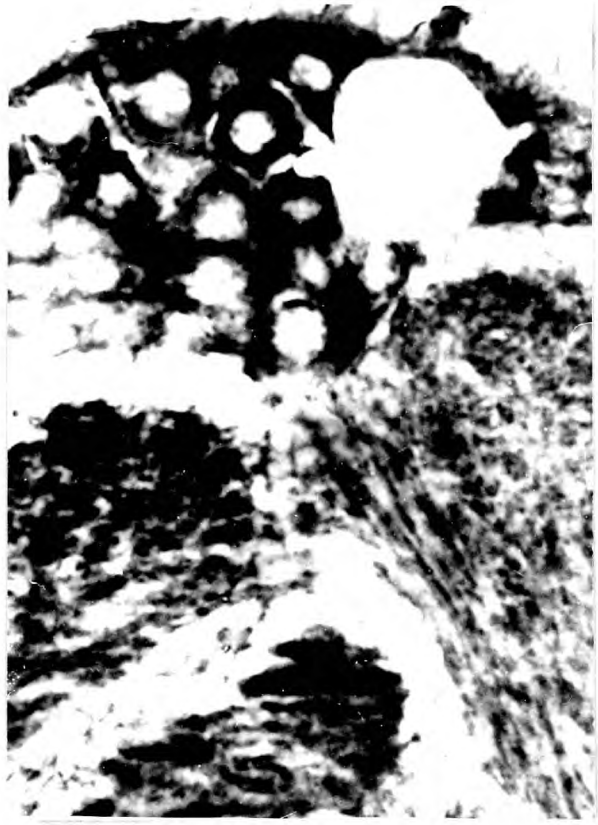
Fig.68: The corpara cardiaca and allata in adult T. granarium.

A: Normal appearance of the glands;
B: Showing artifact due to shrinkage.



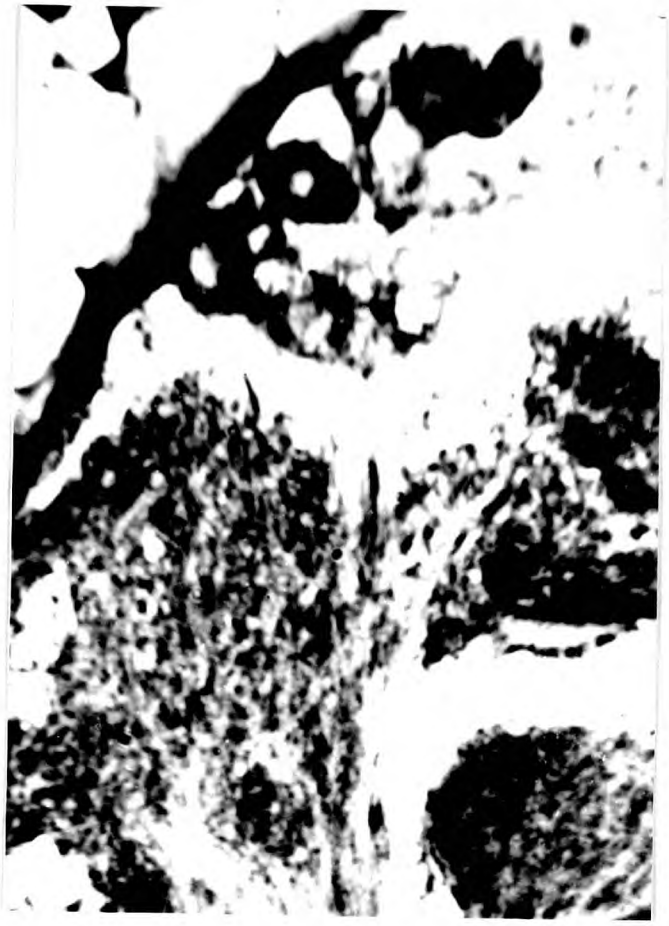
20 u.

Fig. 69: Neurosecretory cells in the brain of an 0-3 hour old adult.



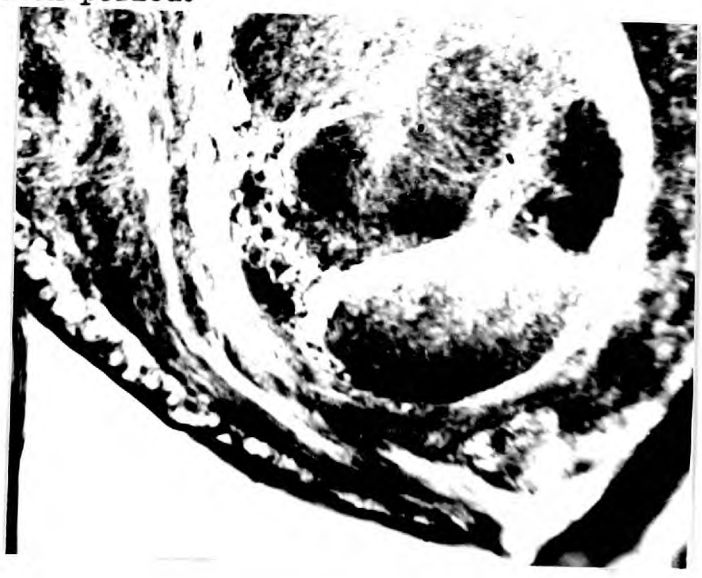
20 u.

Fig.70: Neurosecretory cells in the brain of an adult during the end of maturation.



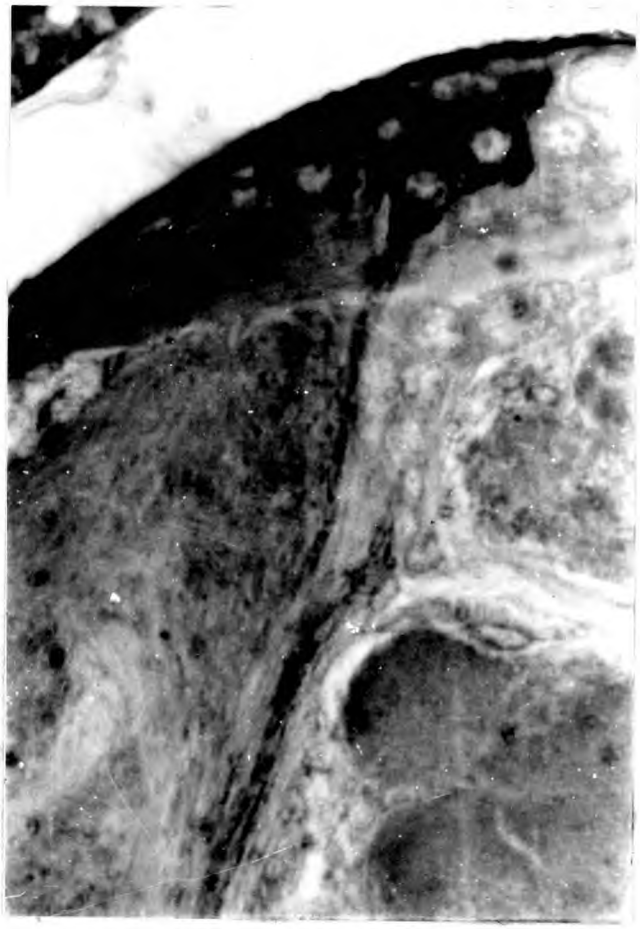
25 u.

Fig.71: Neurosecretory cells in the brain of an adult during the pre-oviposition period.



25 u.

Fig.72: Axons of neurosecretory material in the brainof an adult during the pre-oviposition period.

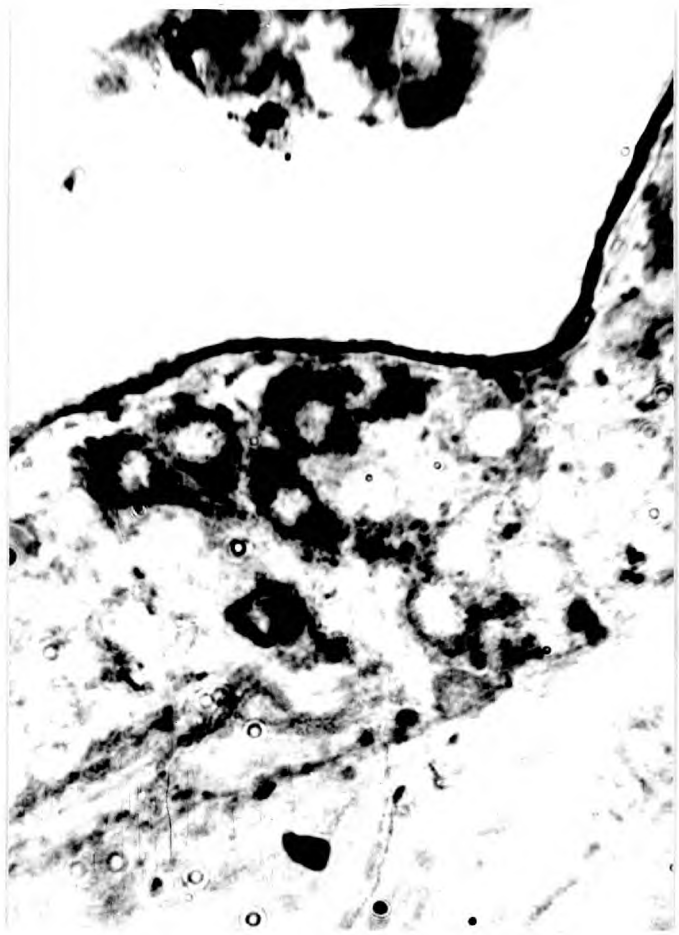


15 u.

Fig.73: Neurosecretory cells and their axons in the brain of a female during oviposition period.

20 u.

Fig. 74: The corpus cardiacum(Cc) and allatum(Ca) in a female during the oviposition period. Notice the brain secretory material entering the glands.

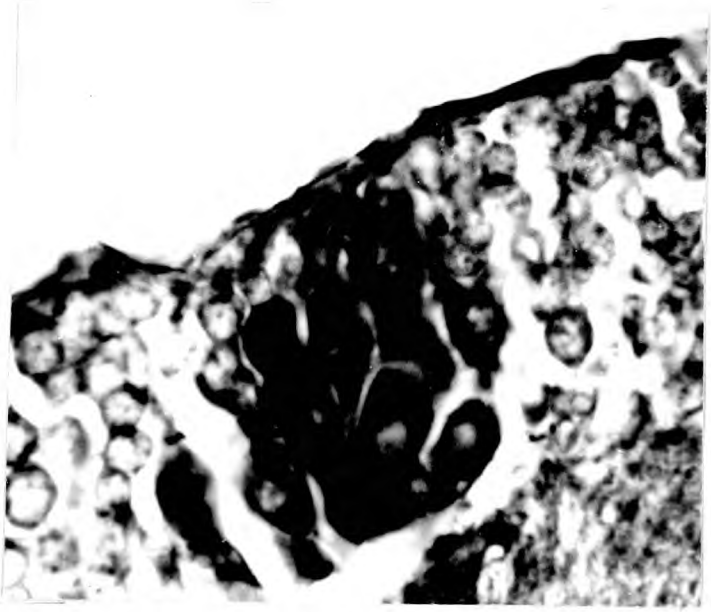


I6 u.

Fig.75: The neurosecretory cells in female during the post-oviposition period.

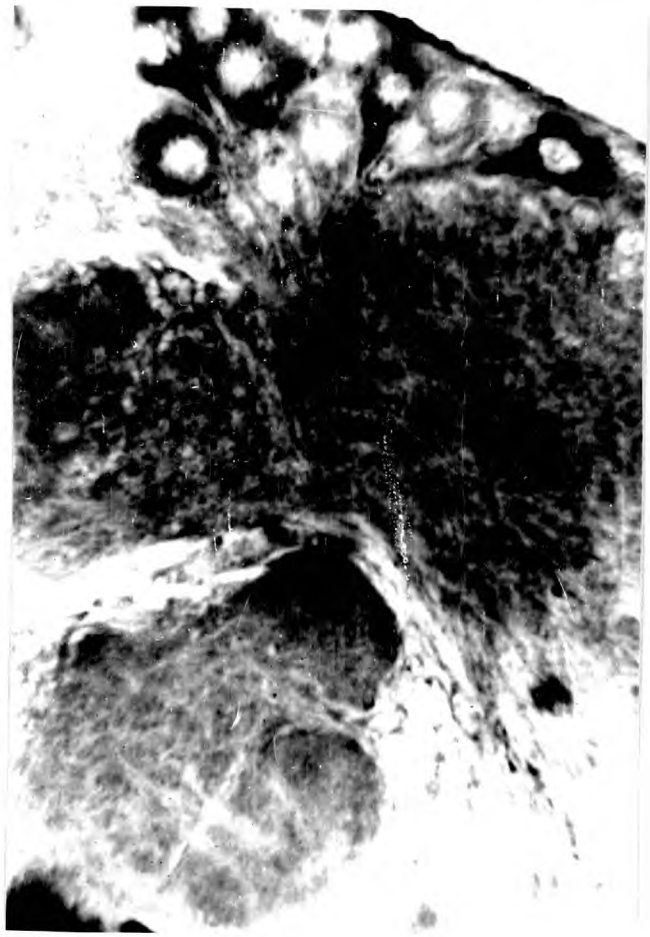
25 u.

Fig.76: The corpora cardiaca and allata in female during the post-oviposition period.



18 u.

Fig.77: The neurosecretory cells in a male during maturation.



24 u.

Fig.78: The neurosecretory cells in a male during copulation.

Fig.79: A diagram showing the correlation of synthesis & release of neurosecretory substance with different physiological phases.

Fig. 79.

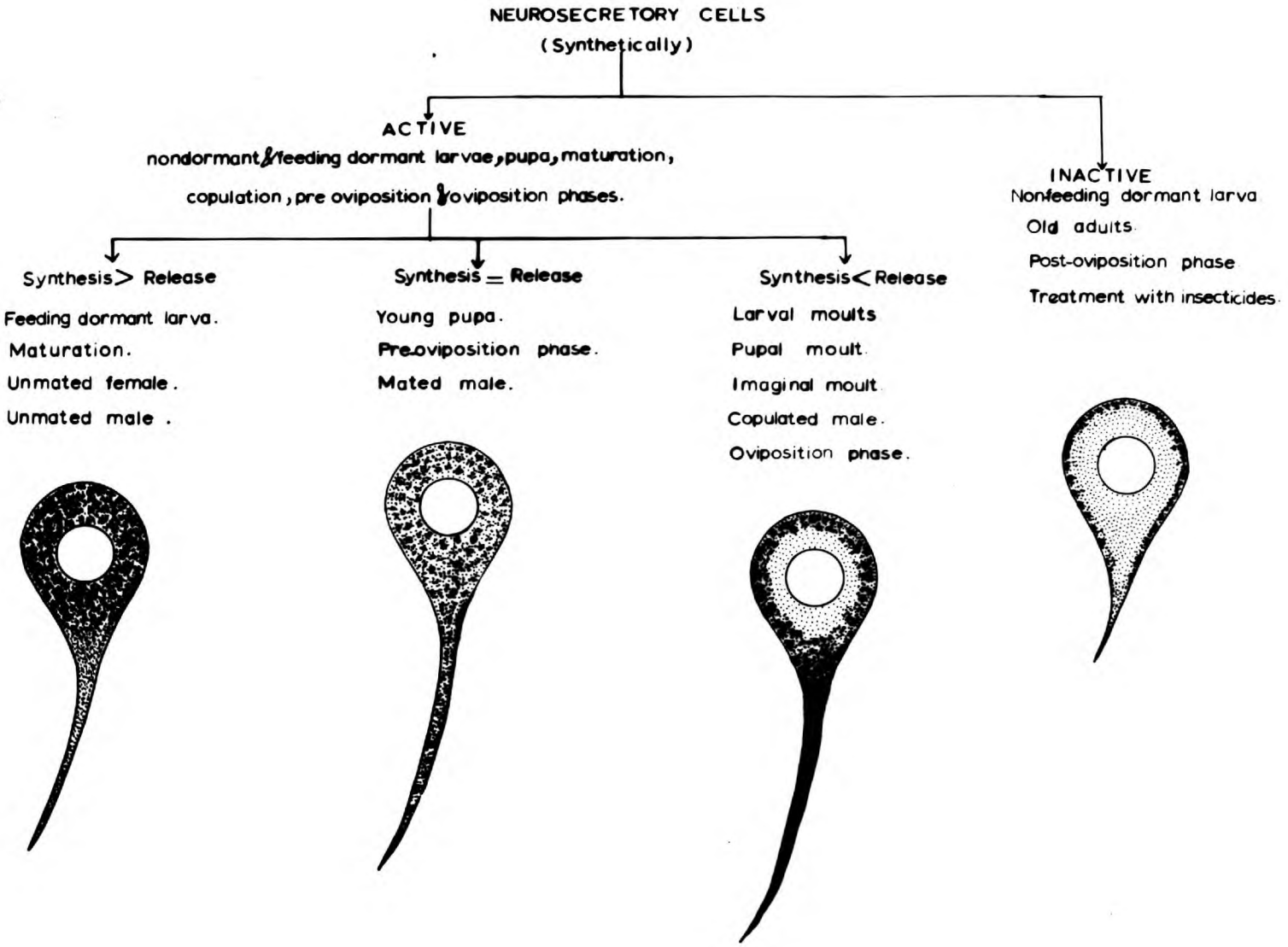

NEUROSECRETORY CELLS
(Synthetically)
ACTIVE
nondormant & feeding dormant larvae, pupa, maturation,
copulation, pre oviposition & oviposition phases.
INACTIVE
Nonfeeding dormant larva
Old adults
Post-oviposition phase
Treatment with insecticides
Synthesis > Release
Feeding dormant larva.
Maturation.
Unmated female.
Unmated male.
Synthesis = Release
Young pupa.
Pre-oviposition phase.
Mated male.
Synthesis < Release
Larval moults
Pupal moult
Imaginal moult
Copulated male.
Oviposition phase.

Fig.80: An ovary after cauterization of the brain neurosecretory cells.

Grm: germarium;
L.ov: lateral oviduct;
Vt: vitellarium.

Fig. 80.

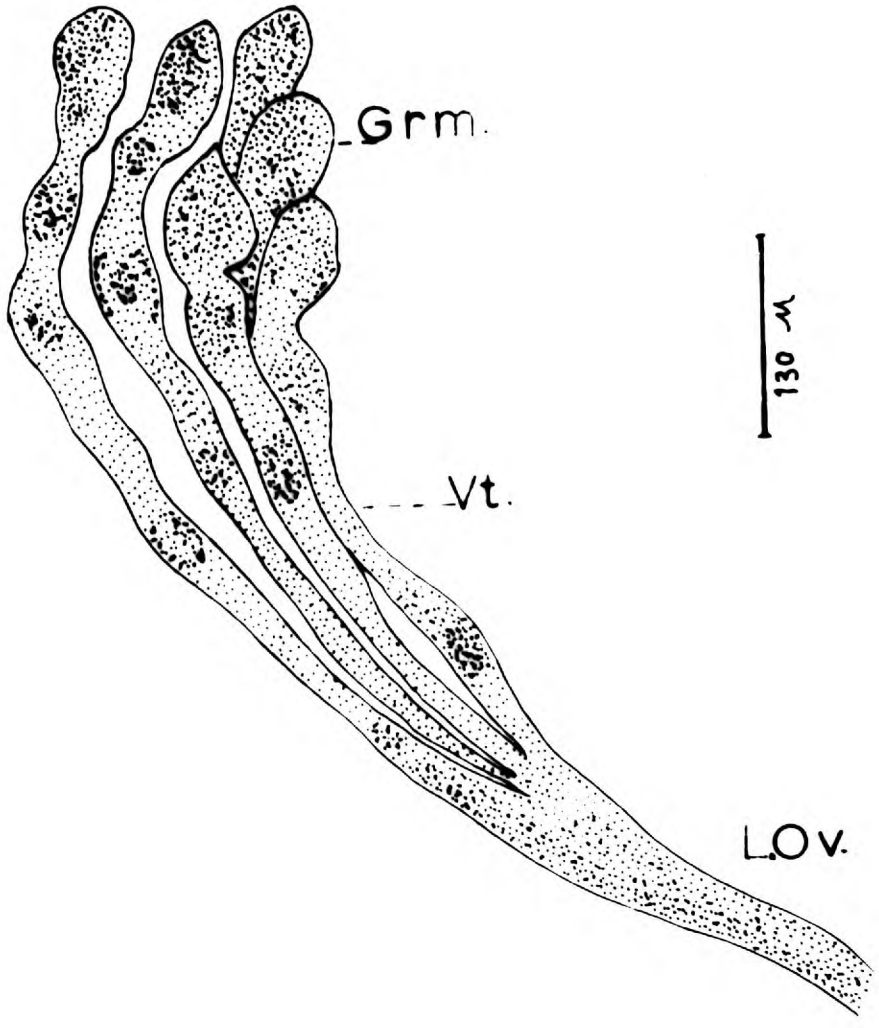
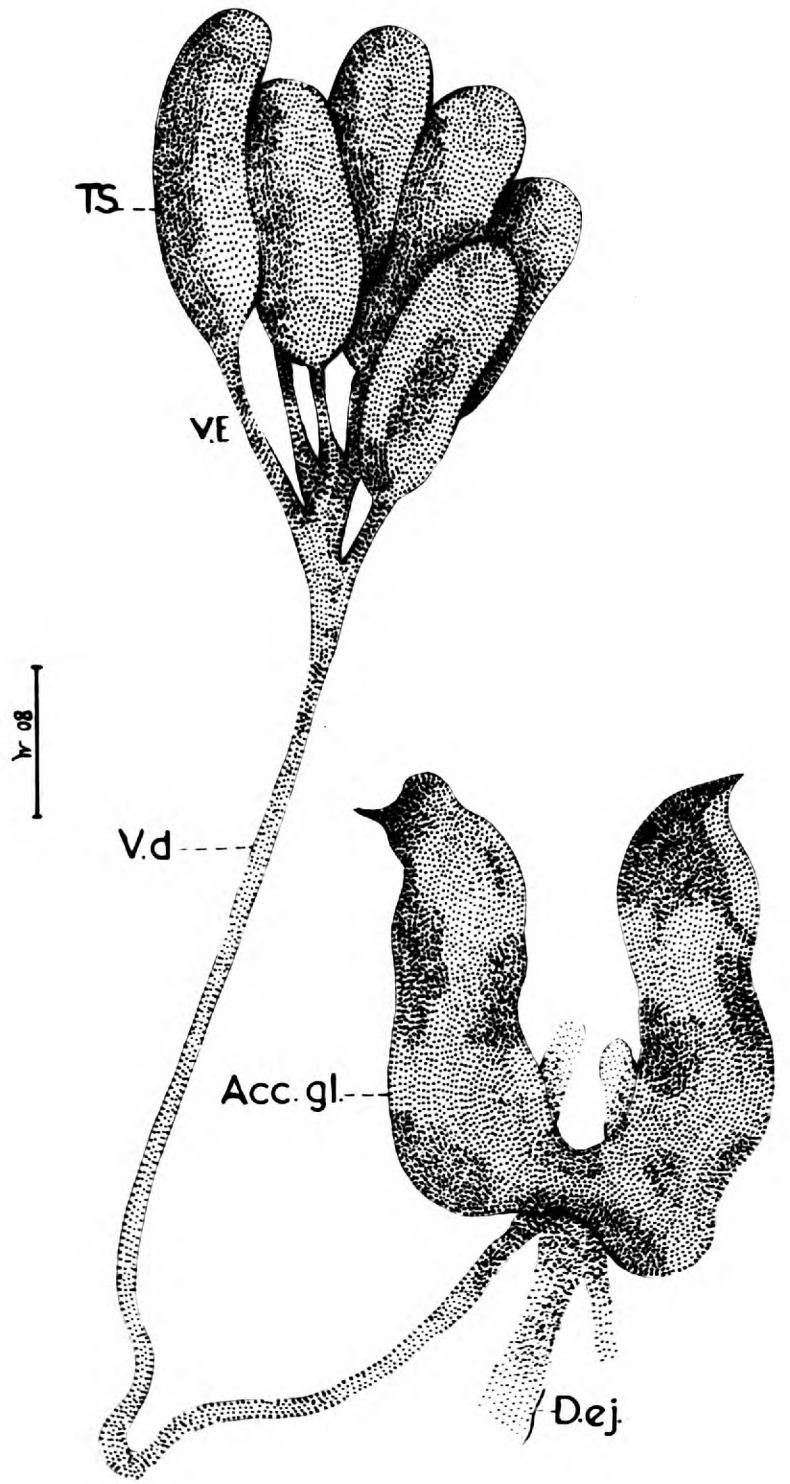


Fig.81: Vitelleria of atrophied ovarioles after cautery of the brain neurosecretory cells.

Fig.82: Testis and the accessory glands after cauterization of the brain neurosecretory cells.

Acc.gl: accessory galnds;
D.ej: ductus ejaculatoris;
TS: testis follicles;
V.d: vas deferens;
V.E: vas efferens;

Fig. 82.



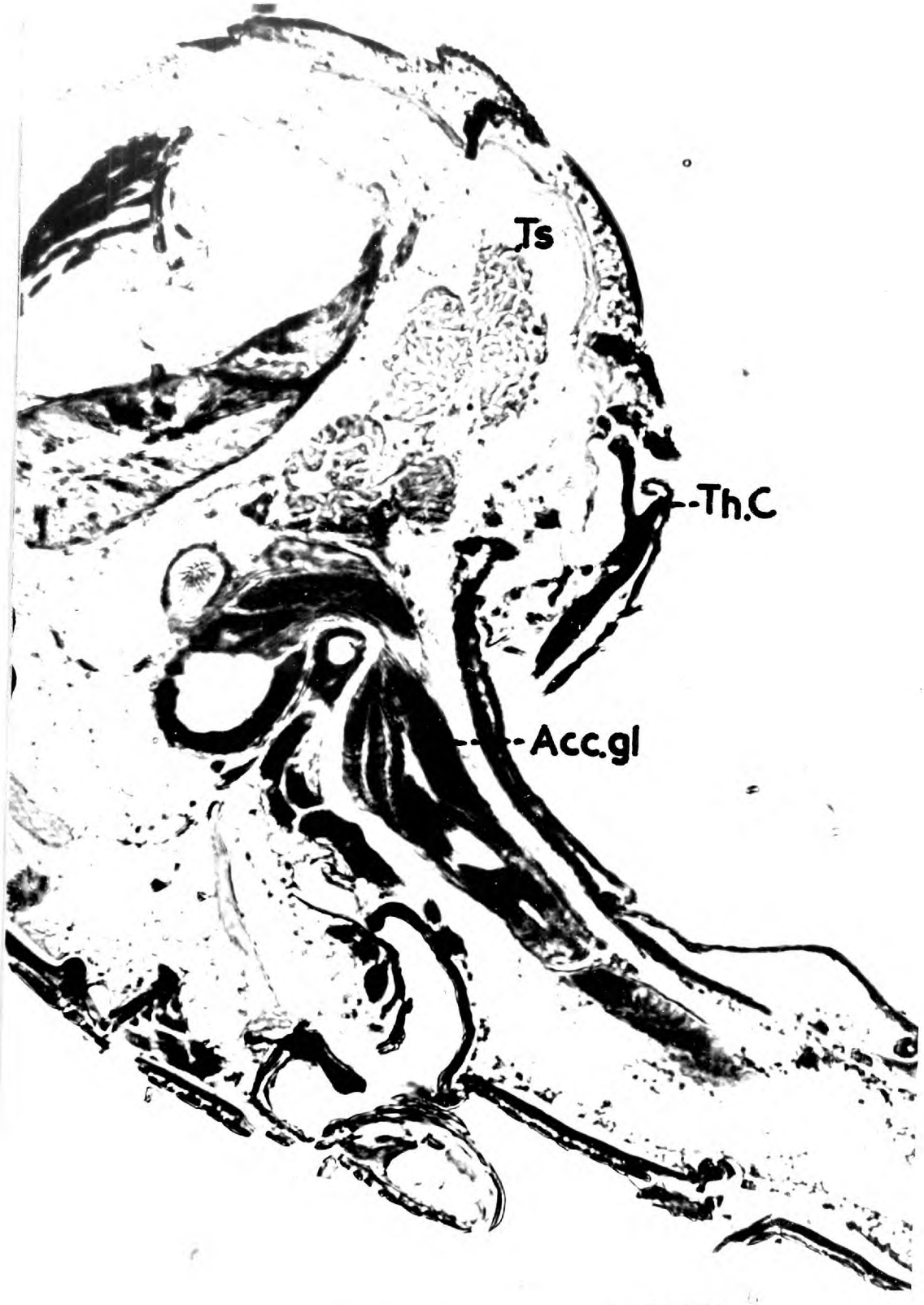
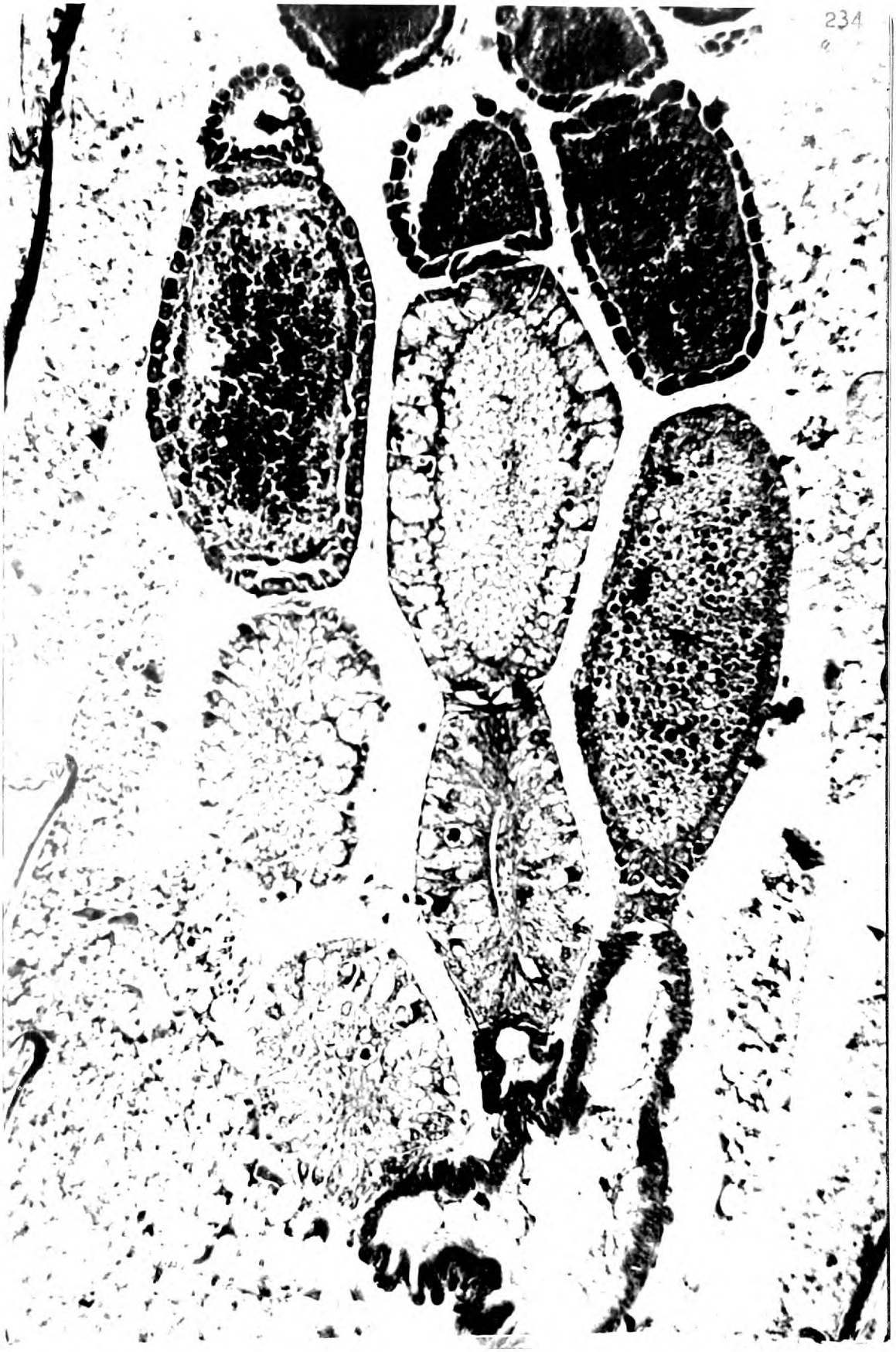
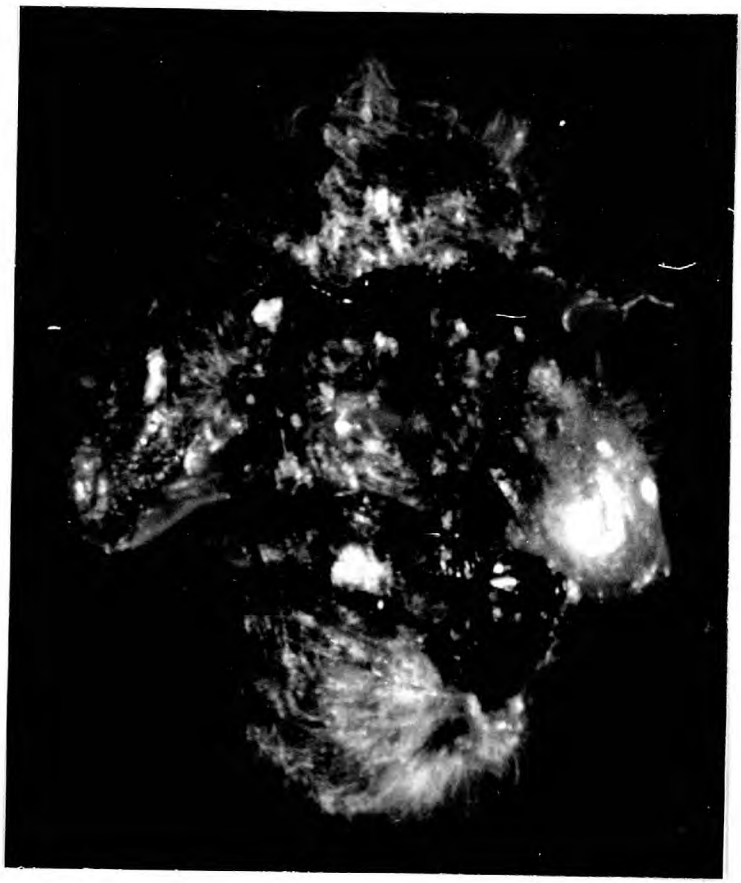


Fig.83: L.S. through the male reproductive system after cautery of the brain neurosecretory cells.
Th.C: thoracic cavity; for other lettering see fig.82.

Fif.84: L.S. through the ovarioles after cautery of the neurosecretory cell in the suboesophageal ganglion in the adult, showing different stages of resorption.



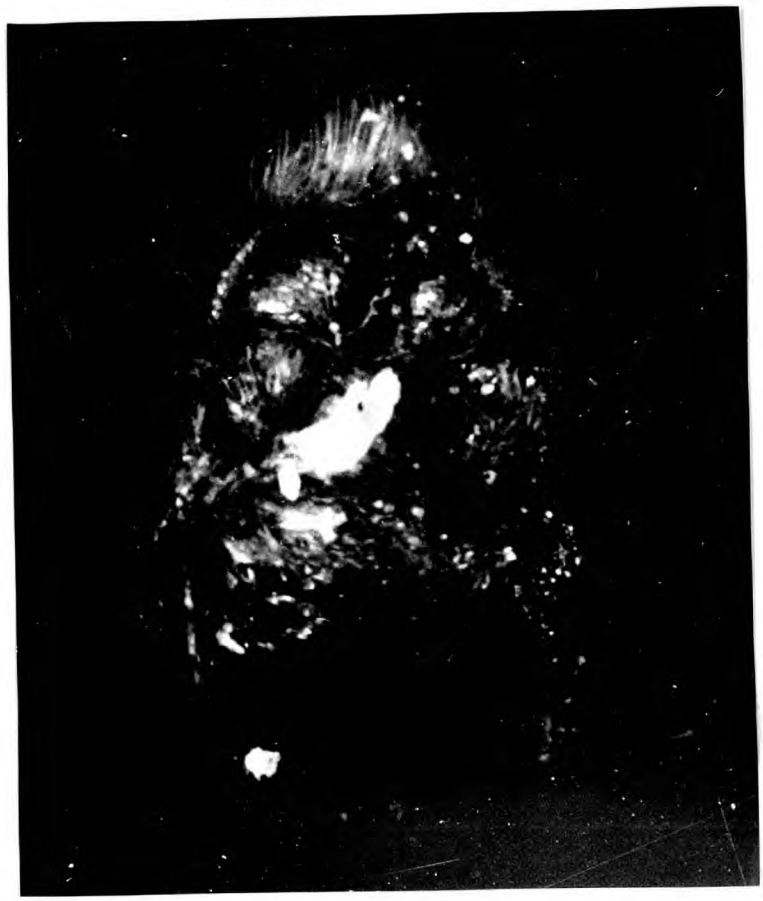


1 mm.

Fig.85: A deformed pupa after treating the larva with insecticides.

(A)

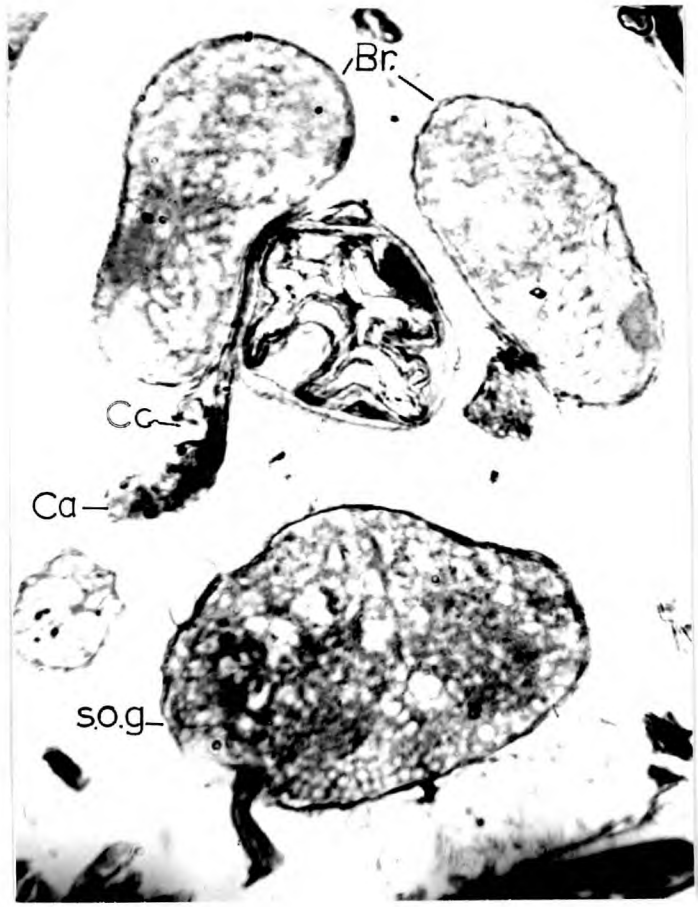
1 mm.



(B)

1mm.

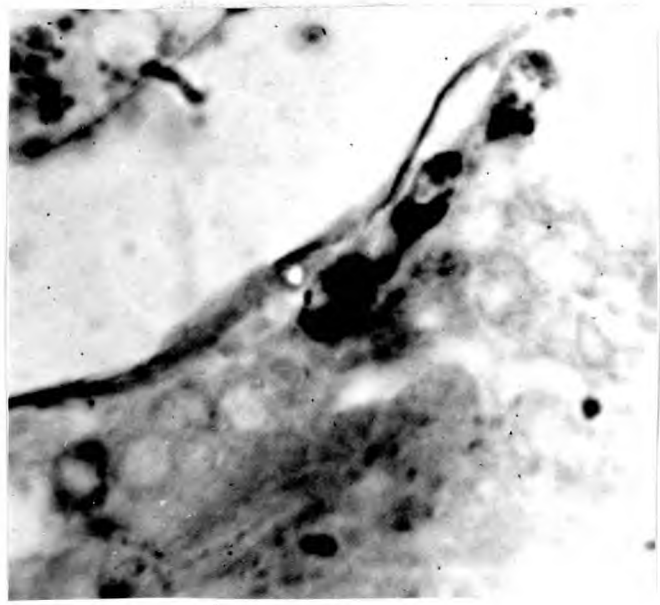
Fig.86: (A)&(B) Deformed adults after treating the larvae with insecticides.



75 u.

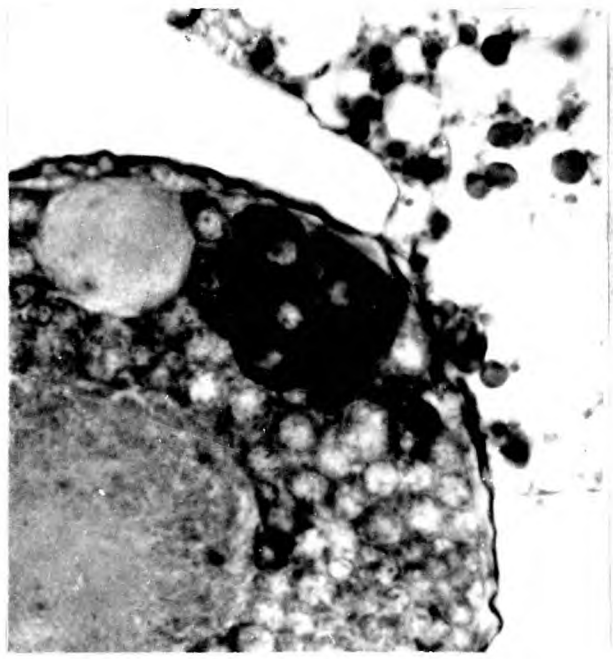
Fig.87: The neuro-endocrine system in paralysed larva.

Br: Brain; Ca: Corpara allata; Cc: Corpara cardiaca; S.o.g: Suboesophageal ganglion.



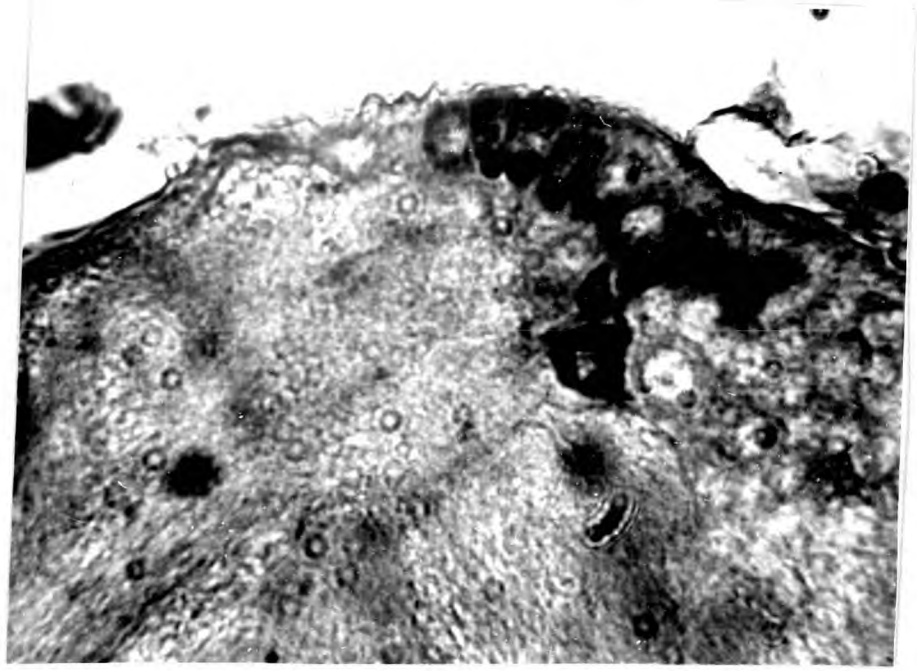
15 u

Fig.88: The neurosecretory cells in paralysed larva.



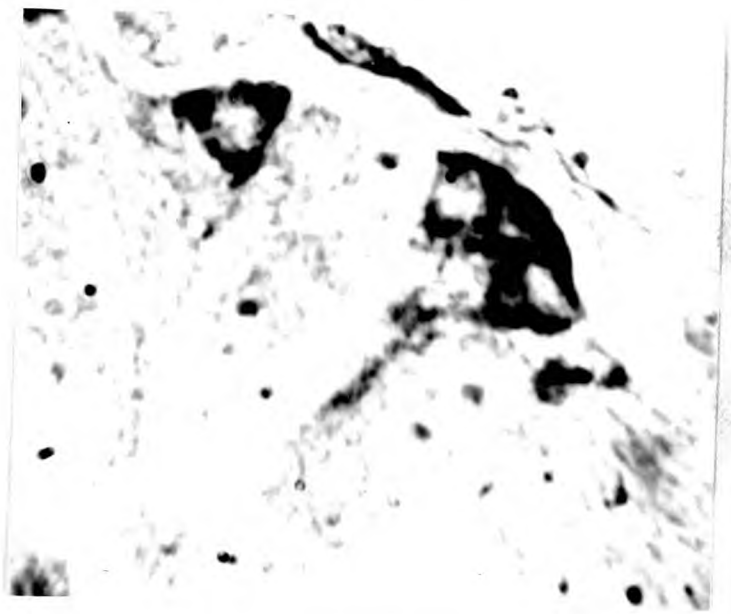
15 u.

Fig.89: The neurosecretory cells in a larva treated with insecticides.



15 u.

Fig.90: Neurosecretory cells in the brain of a pupa after treating the larva with insecticides.



15 u.

Fig.91: Neurosecretory cells in the brain of ovipositing adult after treating the larva with insecticides.