THE HISTOLOGY OF NEUROSECRETION IN THE CEREAL LEAF BEETLE, OULEMA MELANOPUS (LINNAEUS)

Thesis for the Degree of M. S.

MICHIGAN STATE UNIVERSITY
BIBHUTI N. SINGH

1967

ABSTRACT

THE HISTOLOGY OF NEUROSECRETION IN THE
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by B. N. Singh

The histological changes of the neurosecretory system in four different stages of the adult cereal leaf beetle, Oulema melanopus (L.) are described. The heads of the beetles were fixed, dehydrated and embedded by a tetrahydrofuran-parlodion double embedding technique. Three staining techniques were used to differentiate the cell types. The techniques were (1) paraldehyde fuchsin, (2) chrome haematoxylin/phloxine, and (3) alcian blue/phloxine. During diapause the neurosecretory cells of the brain, corpus cardiacum, and the corpus allatum were at a low level of activity. The volumes of the glands and the nuclear volumes of the secretory cells were used to demonstrate activity. At the termination of diapause there was increased activity of the neurosecretory system. During oviposition the neurosecretory system functioned at its highest level. A comparative study of oxygen uptake by the different stages of O. melanopus was undertaken. The lowest amount of oxygen uptake occurred during diapause. There was a direct correlation of the higher level of activity of the neurosecretory cells with higher metabolic activity.

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By

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Entomology

1967

ACKNOWLEDGMENTS

The author expresses his deepest gratitude to Dr. R. A. Hoopingarner for his guidance and encouragement throughout this program of study.

To the members of entomology group at Michigan State University, I offer my thanks for advice and willing assistance at all times during this investigation.

I express my appreciation to the Michigan State University for its financial aid in support of this research.

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INTRODUCTION

Since the introduction of the adult cereal leaf beetle, Oulema melanopus (L.), into Michigan and other parts of the United States, rearing of a large number of beetles has been a problem. The occurrence of an adult diapause in the life-history of O. melanopus is an intriguing problem. The adult beetles undergo diapause from August to early April in Michigan. During diapause the insect spends most of the time in duff, beneath the leaves of wild grasses or other suitable hiding places to avoid unfavorable environmental conditions. Since diapause is an adaptation to withstand extremes of cold, the successful control of those insects having a diapause in the life-history lies in the better understanding and prevention of the diapause stage.

This work was undertaken with a view to learn something about the possible causes of adult diapause, which in the long run might open up possibilities of controlling diapause, thus resulting in natural destruction of the insect population and also eliminating a block in the rearing of this insect for other studies.

As diapause and the resulting failure of growth is associated with profound physiological and biochemical changes, the study of various aspects of the physiology of diapausing insects has attained considerable attention by entomologists. This investigation reports the results obtained from a comparative study of the histological changes of neurosecretory system of the four adult stages of insects vis., pre-diapause, diapause, post-diapause and egg-laying adults.

REVIEW OF THE LITERATURE

Diapause

The term diapause was introduced by Wheeler (1893) to describe a stage in the embryogenesis of the grasshopper Xiphidium ensiferum. In the present day literature diapause is referred to as a state of developmental arrest which occurs in many arthropods during which morphological growth and development are suspended or greatly retarded (Andrewartha, 1952; Lees, 1955). Andrewartha (1952), pointed out that diapause is an adaptation in insects to resist the rigors of the climate.

Diapause is a complex physiological event in its induction, maintenance, and termination. It involves an arrest of growth and a slow down of metabolism, and appears to be the result of changes in the hormone balance within the insect (Beck, 1963; deWilde, 1964; Harvey, 1962; Lees, 1956; VanderKloot, 1955, 1960; Williams, 1946).

Diapause may occur in any stage of the life cycle. Depending upon the species of insect, diapause may be obligatory or facultative. In some species diapause regularly occurs in every generation and is said to be obligatory, although Harvey (1957) has cast some doubt on the validity of the term obligatory diapause. Facultative diapause arises in response to variation in the environment. In typical facultative diapause there may be, within a single growing season, one or more non-diapause generations and then a diapause generation that

passes over the adverse environmental conditions--like winter.

The arrest of growth in arthropods, with a facultative diapause, is governed by the environment. The onset of diapause has sometimes been regarded as a direct response to unfavorable conditions. The effect of day length, temperature, humidity and nutrition in the induction of diapause has been well documented (Lees, 1955).

Temperature

High temperatures tend to avert diapause while low temperatures favor the arrest of growth. This was originally described by Way and Hopkins (1950) in Diataraxia oleracea (L.) and by Lees (1953) in Metatetranychus ulmikoach. Other examples are: Pieris brassicae L. (David and Gardiner, 1952), Chilo suppressalis Walker (Inoue and Kamano, 1957) and Ostrinia nubilalis (Hbn.) (Beck and Hanec, 1960, and Mutchmor and Beckel, 1959). In short-day insects such as the bivoltine race of Bombyx mori (L.) (Kogure, 1933; Freda and Anastasiu, 1955) and in Abraxas miranda Butler (Masaki, 1958), high temperatures promote diapause. High temperature thus appear to eliminate the short-day response. In Grapholitha molestra (Busek), both high and low temperatures prevent diapause (Dickson, 1949). It has been reported that in Colorado potato beetles temperatures within the normal ecological range do not affect photoperiodic induction. Beck (1962) has shown that low temperatures during the scotophase increase the incidence of diapause, whereas high temperatures at that time tend to prevent diapause in O. nubialis (Hbn.). Castro (1964) has found that in the beetle O. melanopus a period of 100 days or more at 4° C. was enough to enable adult beetles to terminate diapause. Temperature

rather than photoperiod was the major factor influencing diapause induction in the tomato horn-worm Protoparce quinquemaculata (Haw.) (Svee, 1964).

There is a definite temperature optimum at which photoperiodic reaction is best manifested, and photoperiodic influences are weakened with departure from the optimum temperature range, which varies considerably from species to species (Danilevsky, 1961).

The Environment and Termination of Diapause

Beck and Hanec (1960) defined diapause as a state of arrested development in which the arrest is enforced by a physiological mechanism. The physiological processes involved in the termination of diapause constitute developmental changes on a biochemical level, and have been termed "diapause development" by Andrewartha (1952).

Two processes, rates of completion of diapause and rates of growth, take place within a particular temperature range and proceed most rapidly at a well defined temperature optimum. It has been observed that insects show great diversity in their thermal requirements for diapause development (Lees, 1955, 1956). Danilyevsky (1949) has studied these physiological requirements in relation to climate and geographic distribution.

Experimental work on diapause development has dealt mainly with low temperature treatments necessary to terminate diapause; the reviews of Andrewartha (1952) and Lees (1955, 1956) discuss many examples of diapause development in eggs, larvae, pupae and adults. In a few cases, termination of diapause has been found to be photoperiodically

induced without a previous exposure of the insects to low temperatures (Paris and Jenner, 1959; Shakhbazov, 1961).

The intensity of diapause, as measured by the length of time required to complete diapause development varies, widely among the species. deWilde et al. (1959) reported that diapause in Colorado potato beetle, Leptinotarsa decemlineata Say, was photoperiodically reversible shortly after the adults displayed diapause behavior, but not a few days later. Hogan (1962) found that the embryonic diapause in the cricket Acheta commodus (Walk.) was more intense after 14 days of incubation at 23° C. than after only 7 days at that temperature.

Photoperiodic termination of diapause has been studied extensively in the European corn borer, O. nubilalis (Hbn.) by McLeod and Beck (1963). Diapause was inhibited, by 14-hour photoperiods in Heliothis zea (Boddie) (Phillips and Newsom, 1966) and 15-hour photoperiods in Protoparce secta (Johannson) (Rabb, 1966). Recently the effect of temperatures on termination of diapause have been reported by Roemhild (1965) in the eggs of Aulocara elliotti (Thomas). Castro (1964) reported that diapause in O. melanopus could be broken by subjecting the hibernating beetles to cold temperatures (ranging from -18° C. to 0° C.). Low temperature has been shown to eliminate diapause in Gryllus campestris (L.) (Fuzeau-Braesch, 1966).

A variety of stimuli such as heat, shocks, and burns terminate the relatively weak diapause in Lucilia (Rouband, 1922). The role of water in relation to diapause has been studied by many workers (reviewed by Lees 1955, 1956). Lees (1956) indicated that dehydration inhibits the secretory activity of some component of the endocrine system, probably the prothoracic glands in the case of larvae.

Hogan (1961, 1962) has reported that urea and certain other ammonium compounds could terminate the embryonic diapause of a cricket, Acheta commodus (Walk). The effect of ammonium compounds on diapausing larvae of European corn borer has been studied by Beck and Alexander (1964).

The inheritance of diapause has been studied in many insects (reviewed by Lees 1955, 1956). The inherited differences always appeared to involve the response to the many agencies which elicit or terminate diapause. The genetics of diapause has been examined in Bombyx mori (L.) (Tanaka, 1953). A genetic mechanism has been implicated in the diapause response of certain other insects, e.g. the European corn borer, Ostrinia nubilalis (Hübner) (Beck and Apple, 1961), the spruce budworm, Choristoneura fumiferana (Clemens) (Harvey, 1957), and certain species of crickets, Gryllus spp. (Bigelow, 1962). Recently Barry and Adkisson (1966) have studied certain aspects of the genetic factors in the control of larval diapause of pink bollworm.

Nutrition

Certain evidence indicates that diapause is often brought on by changes in the diet. The relationship between the fat content of the larval diet and the occurrence of diapause in pink bollworm larvae has been reported by several workers (Fife, 1949; VanderZant and Reiser, 1956; and Bull and Adkissen, 1962). Bull and Adkisson (1960) and Adkisson (1961) demonstrated the importance of photoperiod as a primary factor governing the induction of diapause in the pink bollworm. They suggested that the fat content of the diet appeared to be a secondary stimulus for the induction of diapause.

Photoperiod

Since the classical study of Kogure (1933) on the role of photoperiod in embryonic diapause of the silkworm, Bombyx mori L., there have been many studies on the induction of diapause for survival during hot dry seasons and the induction of diapause for winter survival. The intricate relationship of photoperiod to diapause of insects has been extensively reviewed in recent years by Lees (1956, 1959, 1960); Bunning (1960); Harker (1960, 1961); deWilde (1962) and Beck (1963, 1964). Experiments with long-day insects have shown that high temperatures tend to prevent diapause (deWilde 1962); examples are found in Ostrinia nubilalis (Hbn.) (Beck and Hanec 1960) and Diataraxia oleracea L. (Way and Hopkins, 1950). Diapause development in O. nubilalis is strongly influenced by photoperiod, and diapause can be reinforced in mature larvae through an appropriate manipulation of photoperiod (McLeod and Beck, 1963). The rate of diapause development in O. nubilalis, under conditions of continuous darkness depends upon the rate established by the photoperiods to which the larvae were exposed before being placed in the dark (Beck and Alexander, 1964).

Diapause in O. melanopus could be broken by subjecting the hibernating beetle to cold temperatures for varying lengths of time, followed by exposure to a 16-hour photoperiod (Castro, 1964). Castro (1964) has reported that beetles which had completed full diapause in cool temperatures reproduced at both 12 and 16-hour photoperiods within 6 days, although oviposition at the 12-hour photoperiod was very poor as compared to that of the 16-hour photoperiod. Pupal diapause in the bollworm, Heliothis zea (Boddie) and the tobacco budworm,

Heliothis vireseens (F.) is induced by 10-hour photoperiod and inhibited by 14-hour photoperiods (Phillips and Newsom, 1966). In these cases high temperature (27° C.) counteracted the short photoperiod effect and low temperature (18° C.) counteracted the long-day effect.

Endocrine Processes Involved in Photoperiod and Diapause

A number of physiological processes, including some endocrine functions, have been shown to display rhythmicity in plants, invertebrates, and vertebrates. The cytological evidence in support of the hypothesis that secretory processes may show such rhythmic characteristics are meager (Halberg, 1960; Harker, 1960; Beck, 1963; Bünning, 1963; Wolfson, 1964).

Harker (1956, 1960) has demonstrated in the cockroach that the photoperiod influences a brain center, which in turn controls rhythm of hormone production in the subesophageal ganglion. The photoperiod in aphids (Lees, 1960) and in Chinese oak silkworm, Antheraea pernyi G. (Shakhbazov, 1961) exert its influence directly on the neurosecretory cells of the brain. Adult diapause has been shown to be under photoperiodic control in many cases, e.g. Leptinotarsa decemlineata (deWilde, 1954, 1962), Psyllioides punctulatus Melsheimer (Andersmit, 1961), Coccinella septempunctata (Hodek and Cerkasov, 1961), Anthonomus grandis Boheman (deWilde, 1964 after, Newsom, 1963), and Listroderes obliquus (Klug) (deWilde, 1964 after Newsom, 1963). It has been reported that in these insects the photoperiod controls the function of neurosecretory cells and the corpus allatum. This photoperiodic induction takes place in the central nervous system,

presumably in the brain (deWilde, 1962) which may be considered to be the center of neuroendocrine integration (Scharrer, 1959).

Diapause is currently considered to be caused, primarily, by a failure of the neurosecretory cells to produce brain hormone (or its precursors) (Williams, 1952; Van der Kloot, 1955). The absence of the brain hormone results in an arrest of the production of growth and differentiation hormone (Williams, 1946; Harvey, 1962). Recently, Beck and Alexander (1964) discovered a hormone, "Proctodone", produced by epithelial cells located in the anterior portion of the proctodeum of the larval European corn borer, O. nubilalis. Proctodon was found to be responsible for the activation of the neurosecretory processes leading to the production of the prothoracotropic hormone, thereby constituting a major endocrine component of the physiological processes underlaying diapause development and prepupal morphogenesis (Beck and Alexander, 1964). Beck (1964) has reported the cytological evidence of daily secretory cycles in the proctodone producing epithelial cells, and some of the possible relationships between rhythmic physiological functions and extrinsic photoperiodic signals. The secretory granules occurring in the proctodeal epithelium have been observed to display autofluorescence, leading to the detection of cyclic cell activity (Beck, 1964). The proctodeal secretory activity has been shown to constitute a rhythmic function and this rhythm appeared to be phase-set by the photoperiod (Beck, Colvin and Swinton, 1965).

Diapause and Neurosecretion

The magnitude and the diversities of hormonal control of diapause have been extensively reviewed in recent years (Gilbert, 1964; Fukuda, 1962; Van der Kloot, 1960; Wigglesworth, 1964; Williams, 1958; Fukaya and Mitsuhashi, 1957, 1958, 1961) and are beyond the scope of the present review. The behavioral pattern of neurosecretion in relation to diapause is our main interest, and therefore emphasis will be given only to those aspects of neurosecretion which are directly related to the present investigation, though some general information of the broad prospective of the whole subject will be provided.

Diapause is a complex physiological event in its induction, maintenance and termination. It involves an arrest of growth and a slowing down of metabolism, and appears to be the result of changes in hormone balance within the insect. The endocrine roles in insect growth, metamorphosis, and diapause have been studied intensively during the past two decades (Gilbert and Schneiderman, 1961; Vander Kloot, 1955, 1960, 1961; Wigglesworth, 1954, 1959, 1964; Williams 1952, 1958; Gilbert, 1964). Four endocrine structures are known to be involved in insect growth processes: (1) Neurosecretory cells in the brain which occur in two to three groups--medial, lateral and posterior; (2) corpora cardiaca, a pair of neuroendocrine organs communicating with the brain via two nerves, nervus corporis cardiaci (NCC I) and nervus corporis cardiaci (NCC II); (3) corpora allata, a pair of endocrine glands closely associated with the corpora cardiaca; and (4) the prothoracic glands, which are endocrine organs derived from the epidermis, and in most insects are located in the prothorax

and posterior parts of the head. The structure and form of these endocrine glands vary in different orders of insect.

The sequence of humoral incidents associated with growth and metamorphosis is described as follows: The medial and lateral neurosecretory cells of the brain produce substances that are transferred to the corpora cardiaca via neural axons. The corpora cardiaca release the brain hormone into the blood stream, probably via nerve fibres to the dorsal vessel. Brain hormone activates the prothoracic glands, which then secrete the growth and molting hormone, ecdysone. Ecdysone exerts its physiological effects by stimulating other tissues--such as epidermis and gonads--to initiate differentiation and the molting processes. The corpora allata secrete the juvenile hormone, which plays a role in gonadal development and determines the molting of the insect to a larva, pupa, or adult stage.

Diapause is currently thought to be caused by a failure in the described humoral series of events. Particularly, it is a failure of the brain to produce or to release the brain hormone. The absence of brain hormone results in inactive prothoracic glands and, therefore, no production of ecdysone. Without ecdysone, growth and differentiation ceases, metabolism is suppressed and this arrested state is called diapause (Williams, 1952; Van der Kloot, 1955). The major characteristics of diapause concepts were worked out for pupal diapause by Williams and his colleagues. This concept of humoral sequence is reasonably well fitting in the larval diapause of a number of dipterous and lepidopterous species. Embryonic diapause does not seem to have this kind of humoral basis (Bucklin, 1953; Hasegawa, 1957; Hogan, 1961).

Adult diapause involving gonadotrophic dissociation appears to involve the corpora altata as well as the other endocrine glands (Schroder, 1957; deWilde and deBoer, 1961).

Brain and Neurosecretory Cells

Williams (1946) demonstrated that pupal diapause resulted from the failure of the brain to secrete the brain hormone and that adult development is stimulated only when the brain again becomes active. Williams (1946, 1947, 1948, 1952) found that non-diapause pupal brains implanted into diapause pupae of Hyalophora cecropia caused the termination of diapause and resumption of metamorphic differentiation. Williams suggested that the diapausing brain under the influence of low temperature becomes a "competent brain" which is capable of secreting hormone. At high temperature, this "competent brain" becomes an "active brain," i.e. the neurosecretory cells discharge their hormone(s). Van der Kloot (1955) reported that the diapausing brain is not only endocrinologically inactive but also electrically silent. Recently, Schoonhaven (1962, 1963) has demonstrated that the electrical activity of the brain of several species of insects persists during diapause. There is evidence from work on pupae of other Lepidoptera that some of these animals secrete the brain hormone during chilling (Ichikawa and Nishiitsutsuji-Uwo, 1957; Highnam, 1958), and that the brain is not required in other species (Ozeki, 1954).

The humoral activity of the brain is associated with the presence of neurosecretory cells. Neurosecretory cells are also commonly found in other ganglia of the insect central nervous system. Neurosecretory cells are defined as nerve cells which show cytological evidence of

secretion (Scharrer and Scharrer, 1954). Van der Kloot (1960) stated that neurosecretory cells are 'neuroendocrine' cells which release hormones. Several cytological studies have been conducted on neurosecretory cells to demonstrate their secretory activity (Palay, 1960; Hirsch, 1962; Highnam, 1962; Scharrer and Brown, 1961, 1962; Stiennon and Drochmans, 1961; and Willey and Chapman, 1962).

Different types of neurosecretory cells in the brain have been studied by selective staining techniques. Nayar (1955) distinguished two varieties: A-cells (dark blue with chrome-haematoxylin; purple with aldehyde fuchsin) and B-cells (red with phloxine; green with aldehyde fuchsin). This classification seems most useful and was followed by Johansson (1958); Scharrer (1955); Thomsen (1954), and Highnam (1961). Johansson (1958) also described additional cell types.

In the beetle Galeruca tanaceti (L.), the median neurosecretory cells have 42-46 A-cells, containing varying amounts of granular inclusions at different stages of the life cycle; 10-13 B-cells distributed between the A-cells and 2-5 C-cells (Siew, 1965). Siew (1965) demonstrated that A-cells were small during pre-diapause and diapause phases of this beetle, thus denoting a low level of activity. The nuclear volumes increased during the phase of ovarian maturation and became largest during the phase of oviposition. B-cells also showed an increase in their nuclear volume as the insect approached the oviposition phase. The lateral group of neurosecretory cells in G. tanaceti is composed of 2-3 A-cells and 6-13 D-cells, and these cells show a steady increase towards the oviposition phase, coinciding

with increased activity of the median neurosecretory cells (Siew 1965). Similar types of lateral neurosecretory cells have been described in Iphita limbata Stal. (Nayar, 1955); Calliphora erythrocephala Meig. (Thomsen, 1952); Adelphocoris lineolatus (Geoze) (Ewen, 1962); Dermestes maculatus De Geer (Ladduwahetty, 1962).

In Nebria brevicollis (F.), there are 3 A-cells and 5 B-cells in each group of median neurosecretory cells and 2-4 cells in each lateral group of neurosecretory cells. During pre-diapause, the B-cells are uniform and show lack of stainable material but during the mating season they show distinct granules (Ganagarajah, 1965). Saini (1966) has demonstrated that in Aulacophora foveicollis (Luc.), there are 8 to 10 A-cells and no B-cells in each group of median neurosecretory cells, and 2 B-cells in the lateral group. In this beetle only the median cells show secretory activity and the lateral group of cells does not show secretory activity in either the male or the female.

Siew (1965) demonstrated that in G. tanaceti there were small amounts of A-cell secretion transported along the axons throughout diapause. At the stage of termination of diapause and during the initial stages of ovarian development, the amounts of stainable material were at a maximum along the axons. Towards the time of oviposition the quantity of stainable material was again reduced. In N. brevicollis conspicuous amounts of neurosecretory material were present along the tracts of nervus corporis cardiaci (NCCI) during mating, but negligible amounts were present during pre-diapause (Ganagarajah, 1965).

The median neurosecretory cells of the pars intercerebralis of Iphita limbata seem to release two demonstrable components, probably

representing allatotropin to the corpus allatum and myotropin to the aortic neurohemal site (Seshan and Ittycheriah, 1966).

There is abundant evidence that the activity of the median neurosecretory cells in the brain is a primary requirement for the activity of the corpora allata (Thomsen, 1952; Highnam, 1962). Oöcyte growth is dependent upon factors from the cerebral endocrine system in Schistocerca gregaria (Highnam, 1962), Schistocerca paranensis Burm. (Strong, 1965a), and Tenebrio molitor L. (Mordue, 1965).

Subesophageal Ganglion

Fukuda (1951, 1952, 1953) and Hasegawa (1957) have demonstrated in Bombyx mori that a substance which induces females to lay diapause eggs is liberated by the subesophageal ganglion, probably under the control of the brain. The actual source of hormone appears to be neurosecretory cells in the subesophageal ganglion. Fukuda (1962) believes that the brain stimulates the release of egg diapause hormone from the subesophageal ganglion.

Corpora Cardiaca

Corpora cardiaca are small bodies situated behind the brain and in close association with the aorta. They are fused in some orders of insects. Corpora cardiaca are nervous in origin and contain neurons and chromophil cells which may have a neurosecretory function (Willey and Chapman, 1960; Jenkin, 1962).

In many species of insects, axons running from the brain to the corpora cardiaca show positive staining with common neurosecretory stains (Arvy and Gabe, 1953; Scharrer and Scharrer, 1954; Thomsen, 1954). Histological studies of the corpora cardiaca have been made

recently in many insects including beetles, for example, in the chrysomelid beetle, G. tanaceti (Siew 1965a, 1965b), the beetle A. foveicollis (Saini, 1966), the beetle N. brevicollis (Ganagarajah, 1965), and in the locust L. migratoria (Clarke, 1966). Siew (1965ab) has reported two types of cells in the corpora cardiaca of G. tanaceti, (a) the secretory cells which correspond to the 'chromophile cells' described by Cazal (1948) and (b) the non-secretory cells which correspond to the 'chromophobe' cells of Cazal. These cells are scattered in the matrix of the corpora cardiaca (Siew 1965). Similar types of cells have been demonstrated in the beetle A. foveicollis by Saini (1966). There is an intimate relationship between the axons of the neurosecretory cells and the secretory cells of the corpora cardiaca in G. tanaceti (Siew 1965).

The corpora cardiaca normally function as a store house for the brain hormone and release it into the blood, as pointed out first by Scharrer (1951) in L. maderae. The corpora cardiaca of N. trevicollis does not show accumulation of large amounts of neurosecretory material during different stages of the adult life (Ganagarajah, 1965). In G. tanaceti the corpus cardiacum decreases significantly in volume just after each oviposition, which suggests a role for the gland in oviposition. During post-diapause the volume of the gland increases steadily and attains its greatest size just before oviposition (Siew 1965).

In addition, the corpora cardiaca probably secrete a substance produced by their own cells (Pflugfelder, 1958; Hodgson, 1962). The corpus cardiacum is thought to inhibit the activity of the inhibitory center in the subesophageal ganglion (Milburn et al., 1960).

Corpora Allata

The corpora allata have been implicated in the control of larval, pupal, and adult diapause. The endocrine control of larval diapause in the rice stem borer, Chilo suppressalis Walker, has been extensively investigated by Fukaya and his colleagues (Fukaya, 1951; Fukaya and Mitsuhashi, 1957, 1958, 1961; Mitsuhashi and Fukaya, 1960) and they believe that the corpora allata actively maintain diapause. Histological examinations of the corpora allata indicate activity during diapause and reduced activity at the termination of a diapause. Fukaya and his colleagues proposed that the corpora allata in some manner inhibits the brain or prothoracic glands. Similar hypotheses have also been proposed in the larval diapause of the rice stem borer, Chilo suppressalis (Fukaya and Mitsuhashi, 1961) and the Indian meal moth, Plodia interpunctella (Hübner), (Waku 1960). Highnam (1958) has shown that in the moth Mimastiliae the corpora allata are active during diapause but their secretory activity decreases by the end of diapause. The diapause in the Colorado potato beetle, L. decemlineata, is a result of an inactive corpus allatum (deWilde and Stegwee 1958; deWilde et al. 1959; deWilde 1961; deWilde and deBoer, 1961).

The cells of the corpora allata exhibit cyclic secretory activity and these cycles are correlated with their "juvenile" or gonadotropic effects (Engelmann, 1962; Scharrer and Von Harnack, 1958; Strangways-Dixon, 1961). The necessity of the corpora allata for the maintenance of oöcyte growth after yolk deposition begins has been shown in a number of insects (for detail review see Highnam, 1963). In Coleoptera, it has been reported that the corpora allata are

necessary for oöcyte growth in Dytiscus verticalis (Say) (Joly, 1945) and in Leptinotarsa (deWilde and deBoer, 1961). The corpora allata have been implicated indirectly in the maturation process in Carabus (Joly, 1950) and in Tenebrio molitor L. (Lender and Laverdure, 1964; Mordue, 1965a). In T. molitor, the size of the corpus allatum bears no direct relationship to its activity (Mordue, 1965) and in Schistocerca paramensis Burm., the size and activity of the glands are controlled by the lateral neurosecretory cells or adjacent nervous centers (Strong, 1965).

Many workers have used the corpus allatum volume as an indicator of endocrine activity (Pflugfelder, 1948; Scharrer, 1952; Joly, 1954; Engelmann, 1957; Highnam et al., 1963; Strong, 1964). The corpora allata may, however, secrete without any increase in size, or in fact when they are of subnormal size (Johansson, 1948; Staal, 1961). Mordue (1965a) has indicated that in T. molitor, the corpus allatum volume cannot be used as an absolute criterion upon which to base secretory activity.

In G. tanaceti the histological structure and changes of the corpora allata during different stages of adult life have been studied by Siew (1965). During pre-diapause and diapause stages, the volume of the glands were small and the nuclei were closely packed. The inactive phase was characterized by a low cytoplasmic content, small nuclei of uniform size and an absence of vacuoles. At the termination of diapause, the gland steadily increased in volume and during this phase, the cell differentiated into two types: the peripheral cells which formed the main secretory cells and the central cells. During

the period of oviposition the gland increased to a maximum volume showing marked increase in cytoplasmic content and nuclear volume.

In N. brevicollis during the pre-diapause and diapause period, the corpora allata become enlarged (Ganagarajah, 1965). Similar types of changes in the corpora allata have been observed in the beetles Dermestes maculatus De Geer (Ladduwahetty, 1962) and A. foveicollis (Siani, 1966).

Diapause and Respiration

Oxygen consumption during diapause often falls to little more than one-tenth of the value found in growing and reproducing insects. These trends in the amount of respiration have been followed in the Platysamia pupa (Schneiderman and Williams, 1953) and in the tenebrionid beetles Anatolica and Opatrum (Edelman, 1951).

Respiration in diapause is marked by a complete cessation of anabolic processes and a consequent sharp drop in the energy demands of the tissues (Harvey, 1962). It might be expected that the reduction of energy demands during diapause be marked by a precipitous fall in the rate of respiration. In the eggs of Melanoplus spretus (Walsh) during diapause, respiration is maintained at about one-third or one-quarter of that shown by developing eggs at the same morphological stage (Bodine, 1929). The respiratory quotient (RQ) in the developing grasshopper egg drops rapidly from near unity to approximately 0.6 in pre-diapause, at which level it remains fairly constant until diapause is terminated. In early post-diapause the RQ shows a slight decline followed by a rise and leveling off at the time of hatching (Boell, 1935).

The respiration of many diapausing insects is remarkably insensitive to cyanide, carbon monoxide, and other inhibitors of cytochrome oxidase. At the onset of diapause in Hyalophora cecropia, respiration drops to one-fiftieth of its previous rate, and at this point the insect becomes resistant to the effects of cyanide and carbon monoxide. The insensitivity to cyanide and carbon monoxide during diapause was originally attributed to a deficiency in cytochrome oxidase (Schneiderman and Williams, 1954; Schneiderman, 1957), but later work has shown that the insensitivity is due to the presence of a large excess of cytochrome oxidase in relation to cytochrome C (Harvey and Williams, 1958; Kurland and Schneiderman, 1959; Shappirio, 1960; Gilbert and Schneiderman, 1961; Harvey, 1962).

MATERIALS AND METHODS

The beetles used in this study were collected from the field and reared at the Entomology Experimentation Station of Michigan State University under controlled condition of temperature (24° C. - 27° C.), humidity (50% - 60%) and daily period of illumination (16-hour photoperiod). Different stages of the adult beetles were obtained from the above source whenever needed and were either used immediately after bringing them in laboratory or stored if necessary under similar controlled conditions in a controlled environment chamber. Diapause and post-diapause stages were stored at 4° C.

Pienaar's (1955), methanol-chloroform-propionic acid (6:3:2) fixative was used. Different stages of the beetles (pre-diapause, diapause, post-diapause and egg laying) were killed simultaneously by putting them into the above fluid. After a few minutes they were decapitated and the heads were then put into fresh solution of the fixative. There was an initial vacuum infiltration under reduced pressure which insured rapid penetration of the fixative. Fixation was continued for 24 hours. The heads were then dehydrated in two changes of tetrahydrofuran for a total of four hours. The embedding was done following Salthouse's (1958) double embedding technique. Tissue blocks were made following the usual procedures and sections of 9-10 microns were made on a rotary microtome. Batches of ten slides

were stained at a time to insure the uniform treatment of both experimental and control sections.

Three different staining procedures were employed to stain the slides, as an evaluation of these methods and to determine the one yielding the most satisfactory results for the insect species concerned in this study.

(I) Most of the slides were stained with paraldehyde fuchsin (PF) and counterstained with light green following Cameron and Steele (1959). Aldehyde fuchsin was prepared according to Gabe's method (1953). De-paraffinized and hydrated sections were oxidized in Gomori's fluid (1941) (0.15 gm. of $KMnO_4$ in 50 ml. of H_2O containing 0.1 ml. of concentrated H_2So_4) for one minute.

(II) Some of the slides were stained with chrome haematoxylin followed by a counterstaining with phloxine as described by Gomori (1941). De-paraffinization and hydration were done following the usual procedure of Gomori but as regards the staining Gomori did not mention precisely the duration period in chrome-haematoxylin stain (CHP). However, after several trials most satisfactory results were obtained by 15 to 18 minutes staining in chrome-haematoxylin solution.

(III) Some of the slides were stained with alcian blue and counterstained with phloxine. Sections were passed through a graded series of alcohols and brought to water and then oxidized with acidified $KMnO_4$ (as described above (I)) for one minute. After a brief rinse in SO_3 solution (2.5% sodium bisulfite) and distilled water the tissues were stained with 0.2% solution of alcian blue stain in 3% of acetic acid for 12 to 15 minutes, rinsed with distilled water and counterstained with 1% aquous solution of phloxine for 3-4 minutes, rinsed

and immersed in 5% phosphotungstic acid solution for 30 to 50 seconds, rinsed and differentiated in 95% alcohol and then dehydrated and mounted in permount following the usual procedure.

Some adult beetles that were in diapause or post-diapause were exposed to a short-day photoperiod (8 hrs.) for two days. Beetles of these two stages were taken from the environment chamber in the morning (9 a.m.), noon (1 p.m.) and afternoon (4 p.m.) to demonstrate their proctodeal secretory activity. At each time beetles were dissected in fixative and their proctodeums freed from tracheae and fat bodies and continued fixation for 24 hours. Tissue were embedded in 61° C tissuemat and sections of 4-7 microns thickness were cut on a microtome. Staining was done by the paraldehyde-fuchsin technique of Cameron and Steele (1959). Sections were examined under a fluorescence microscope by using a UG-1 2-mm. exciter filter and an Euphos barrier filter. Fresh proctodeums from living beetles were dissected out under insect saline and excess fat and tracheae removed. They were then split laterally, mounted flat in saline on a microscope slide, and covered with coverslip, and examined with a fluorescence microscope with the above described conditions.

RESULTS

Histology of the Neurosecretory System

The neurosecretory cells in the brain of Oulema melanopus (L.) could be separated into three main groups viz. median, lateral, and anterio-ventral. The median group of neurosecretory cells were subdivided into one median-dorsal and two median-lateral groups (Fig. 1). They were present in the dorsal-median aspect of the pars intercerebralis of the protocerebrum. The axons of these median groups run anteriorly and ventrally. In serial sections, cut sagittally, there was no distinct demarcation between the two groups of median-dorsal cells, which for convenience were considered as one group. There were about 22-26 median neurosecretory cells in the brain, containing varying amounts of granular inclusions at different stages of the adult life cycle. These neurosecretory cells were shown to contain both A- and B-cells. The alcian blue/phloxine (ABP) technique was very useful in demonstrating A and B-cells.

Lateral Group of Neurosecretory Cells of the Protocerebrum

The lateral group of neurosecretory cells, one in each cerebral hemisphere, was located dorsal-laterally in the protocerebral lobe of the brain (Fig. 1). Both A and B type of cells were present but it was not possible to count the number. There were, however, more

A-cells than B-cells. The staining affinities and cytology of the A and B-cells were similar to A and B-cells of the median group of neurosecretory cells.

Anterio-ventral Group of Neurosecretory Cells in the Protocerebrum

It was observed that in the anterior-ventral part of the brain, there was a group of 2-4 neurosecretory cells (Fig. 1). This group could be considered as a anterio-ventral group of neurosecretory cells. Sections stained in paraldehyde fuchsin indicated two A-cells and two B-cells. In some sections it was very difficult to locate these cells.

The Staining Reactions of the Cytoplasm of the Neurosecretory Cell Types of O. melanopus using the Three Staining Techniques

In the sections stained in paraldehyde fuchsin, most of the cells in the median and lateral groups of neurosecretory cells, were yellowish-orange with dark orange inclusions. Some were light yellowish-green with light green inclusions. Cytological structures of the orange cells were more distinct than those of the yellowish-green cells. In some cases deep purple inclusions were also observed in the yellowish-orange cells. The yellowish-orange cells were considered as A-cells and yellowish-green cells as B-cells. Sometimes it was very difficult to find the B-cells due to their light staining. Sections stained with chrome-haematoxylin/phloxine method showed that all the neurosecretory cells were purple with deep purple inclusions. The alcian blue/phloxine technique also stained neurosecretory cells satisfactorily. Most of the median and lateral neurosecretory cells stained blue with deep blue inclusions and were probably A-cells.

There were some red cells with red inclusions which were considered as B-cells. Nuclei of the blue cells contained 1-3 red-rose colored droplets besides the blue chromatin. In all preparations, the majority of the median and lateral neurosecretory cells were of the "A type" while a few were similar to "B type" cells.

Neurosecretory Pathways

The axons from the median group of neurosecretory cells passed anteriorly and then curved posteriorly and decussated, entering the nervus corporis cardiaci I (NCC I). Thus each corpus cardiacum was innervated by axons from the opposite median group of neurosecretory cells. The axons from the lateral group of neurosecretory cells probably passed directly into the nervus corporus cardiaci II (NCC II) without decussating. These axons were very close to NCC I and it was not possible to distinguish them. Secretory cells were also observed in the NCC I and NCC II (Fig. 9, 10, 11, 12).

The Corpus Cardiacum

The corpus cardiacum consisted of two types of cells (Fig. 8-12). The first type considered to be secretory cells probably correspond to the 'chromophile cells' described by Cazal (1948). These cells stained yellowish-green with paraldehyde fuchsin and purple with chrome-haematoxylin/phloxine and blue with alcian blue/phloxine. Cytoplasmic inclusions and nuclei were very clear when the section was stained by the chrome-haematoxylin/phloxine method. Most of these cells were located in the cortex region of the corpus cardiacum. The second type of cells were considered to be non-secretory cells and

they were scattered in the matrix of the corpus cardiacum. Some of these non-secretory cells were found to be present between the secretory cells of the corpus cardiacum. These non-secretory cells probably correspond to the 'chromophobe cells' of Cazal (1948). These cells stained orange with paraldehyde fuchsin, red with chrome-haematoxylin/phloxine and alcian blue/phloxine. They were smaller than the secretory cells. The axons from the NCC I and NCC II seemed to intermingle in the anterior portion of the corpus cardiacum.

The Corpus Allatum

The corpus allatum was invested with a thin connective membrane which was continuous with that of the corpus cardiacum (Fig. 10). The complete structure of the corpus allatum appeared to be a compact ball like body. Two types of cells were observed in the corpora allata (Fig. 18, 19). Sections stained in chrome haematoxylin/phloxine showed two types of cells: (i) purple cells with purple inclusions, and (ii) red cells with red-violet inclusions. There were only a few red cells. In the alcian blue/phloxine staining technique there were blue cells with blue inclusions and red cells with red inclusions. The blue cells also contained 1-3 red, droplet like granules within their nuclei besides the blue chromatin. The nuclei of the purple and blue cells were very distinct and had deep purple and blue chromatin. These cells were considered to be the main secretory cells of the corpora allata, and the red cells did not show a distinct nuclear structure or much secretory activity. Sections stained with paraldehyde fuchsin also showed two types of cells, yellowish-green cells with granular inclusions and yellowish-orange

cells with dark orange inclusions. Mitotic activity in the cells was not observed. Neurosecretory material from the brain was not observed in this gland. In the inactive gland the nuclei were closely packed together and distributed homogeneously in the gland. In the active gland the nuclei were distributed heterogeneously.

Histo-Physiological Changes in the Neurosecretory System of Oulema melanopus (L.) During Different Stages of the Adult Life Cycle

The Neurosecretory Cells

During the pre-diapause and diapause stages of the beetle, there was no difference in the shape or in the cytological structure of median or lateral neurosecretory cells. Sections stained with alcian blue/phloxine showed that during diapause the neurosecretory cells were comparatively smaller, contracted, and of irregular shape (Fig. 3) --denoting a low level of activity. In pre-diapause the cells were also small, but not contracted (Fig. 2) and most of the cells were of uniform shape. Similar results were also found in the posterior neurosecretory cells. In the post-diapause period there was an increase in the size of the neurosecretory cells and the cells started losing their contracted appearance (Fig. 4). During the egg laying stage neurosecretory cells (Figs. 5, 6, 7) attained their maximum size and the cytoplasmic inclusions became more distinct in comparison to other stages. Their nuclear volume also increased during oviposition, suggesting a higher level of activity during this phase.

The Corpus Cardiacum

During diapause and pre-diapause, the corpora cardiaca were

small. The cytoplasmic inclusions and nuclei of their cells (Figs. 8, 9) were not as distinct as they were in the egg-laying stage. During post-diapause the volume of the gland increased very slowly and attained its full size during oviposition. The secretory 'chromophile cells' became enlarged corresponding to the size of the gland (Fig. 10-12). Their nuclear volumes increased slightly during oviposition. Certain small cells (1 to 3) were also observed in the axon of NCC I (Figs. 9, 10, 11 and 12), from the brain, passing into the corpus cordiacum (Figs. 9, 10). These cells appeared to be secretory cells. In some sections a non-secretory type of cell (1-3) was also observed in the NCC I along with the secretory cells. It was assumed that these cells were probably A and B-cells of the brain neurosecretory groups passing into the corpus cardiacum through NCC I. The corpora cardiaca did not show an accumulation of large amounts of neurosecretory material during the different stages, although some material was found along the anterior margin and at the center of the gland.

The Corpora Allata

The corpora allata showed much variation both in size and in histological appearance. During pre-diapause (Figs. 13 & 14), the corpora allata remained small and were presumably inactive. The small volume of the gland continued during diapause (Figs. 15 & 16). The nuclei were closely packed, being distributed homogeneously in the matrix of the gland. The inactive phase was characterized by a low cytoplasmic content, absence of vacuoles, and small nuclei of uniform size. The size of nuclei in pre-diapause and diapause varied from 3.4 μ to 4.5 μ.

During post-diapause (Fig. 17) the gland increased steadily in its volume. This was accompanied by a marked increase in cytoplasmic content and nuclear volume. The size of the nuclei varied from 4.2μ to 6.5μ. At oviposition (Figs. 18 & 19) the gland increased to its maximum volume accompanied by a marked increase in its cytoplasmic content. It was observed that the peripheral cells of the corpus allatum usually increased in size far faster than those of the central region of the gland. The peripheral cells of the corpus allatum were assumed to form the main secretory cells. The gland showed a characteristic heterogeneous distribution of nuclei and the presence of vacuoles during oviposition, and the nucleolus and chromatin within the nucleus were more distinct. The size of nuclei increased up to 9μ. The increase in size of the gland was attributed to the increase in both the nucleus and cytoplasm.

Proctodone Evaluation

Stained microscopic sections through the anterior region of the proctodeum showed only a few cells with purple granular inclusions. These granules were very scarce in diapause and post-diapause beetles. Often it was not possible to find these cells in proctodeal tissue. These cells are considered to be secretory cells in the European corn borer (Beck, 1964, 1965). These cells in diapause and post-diapause did not show fluorescence when stimulated with the recommended ultra violet wave lengths. The proctodeal tissues mounted in saline were also negative in that they did not fluoresce.

Oxygen Consumption of the Adult Cereal Leaf Beetle

Diapause is characterized by cessation of most anobolic processes, resulting in a sharp decrease of the demand of energy in the diapausing insects. Since in diapausing insects most anobolic processes are stopped, resulting, therefore, in less consumption of oxygen. The rate of respiration as reflected in O_2 consumption was thought to be worth studying in the different stages of the cereal leaf beetle.

Respiration of the different stages of the adult beetle O. melanopus was measured in a Warburg respirometer following the usual manometric techniques (Umbreit, Burries, Stauffer, 1959). The insects were carefully put into the side arm of the Warburg flask to prevent their creeping down into the KOH of the center well. Two insects were kept in each flask and duplicate observations of each stage were made. All measurements were carried out at 25° C. The experiments were replicated and similar results were obtained each time (Table 1).

TABLE 1

OXYGEN CONSUMPTION OF THE CEREAL LEAF BEETLE

Stage	Dry Wt. (mg.)	μl O_2/mg. dry wt./hr.
Pre-diapause	7.4	3.37
Diapause	6.0	0.78
Post-diapause	6.4	1.00
Egg-laying	6.6	2.81

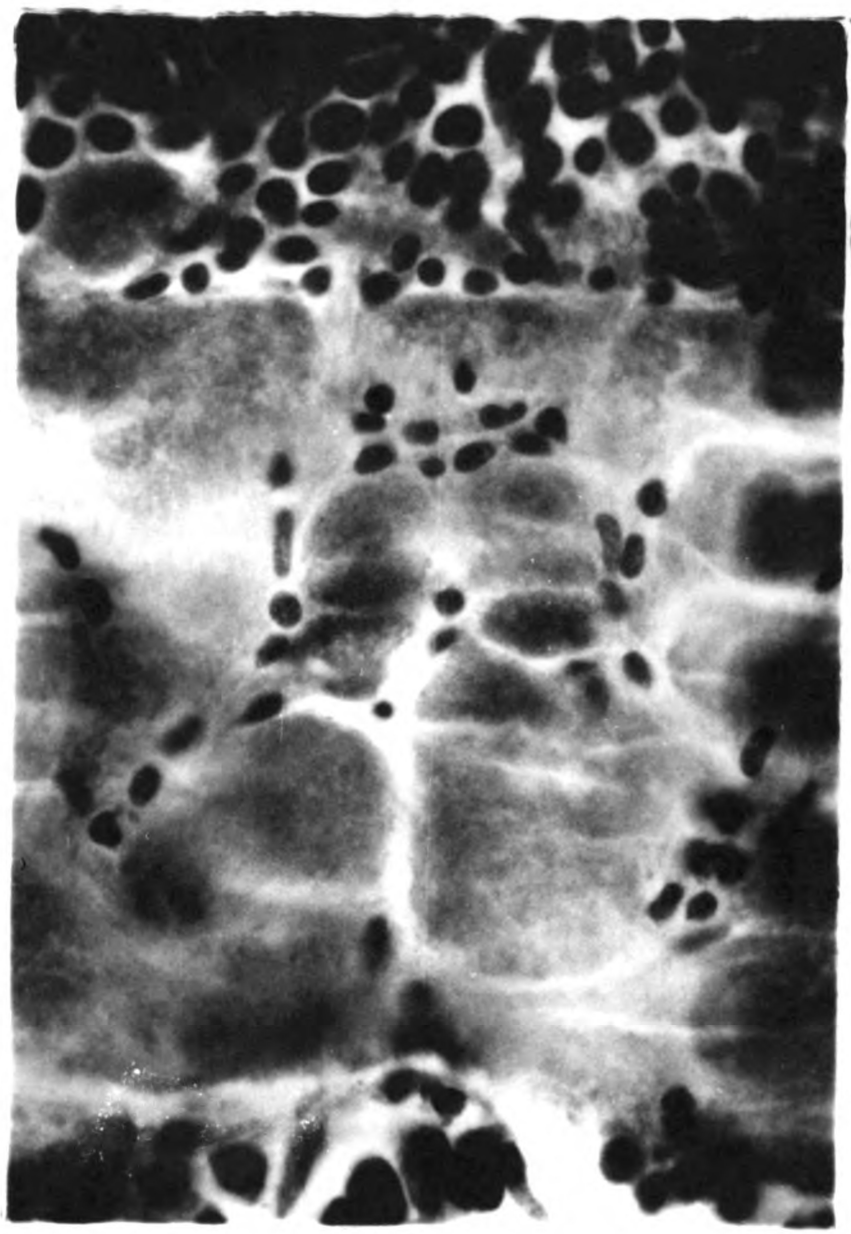

Fig. 1. Sagittal section of the brain of male O. melanopus in pre-diapause showing the distribution of median, lateral and posterior groups of the neurosecretory cells in protocerebrum.
Stain--Paraldehyde fuchsin. Magnification 1100 X.

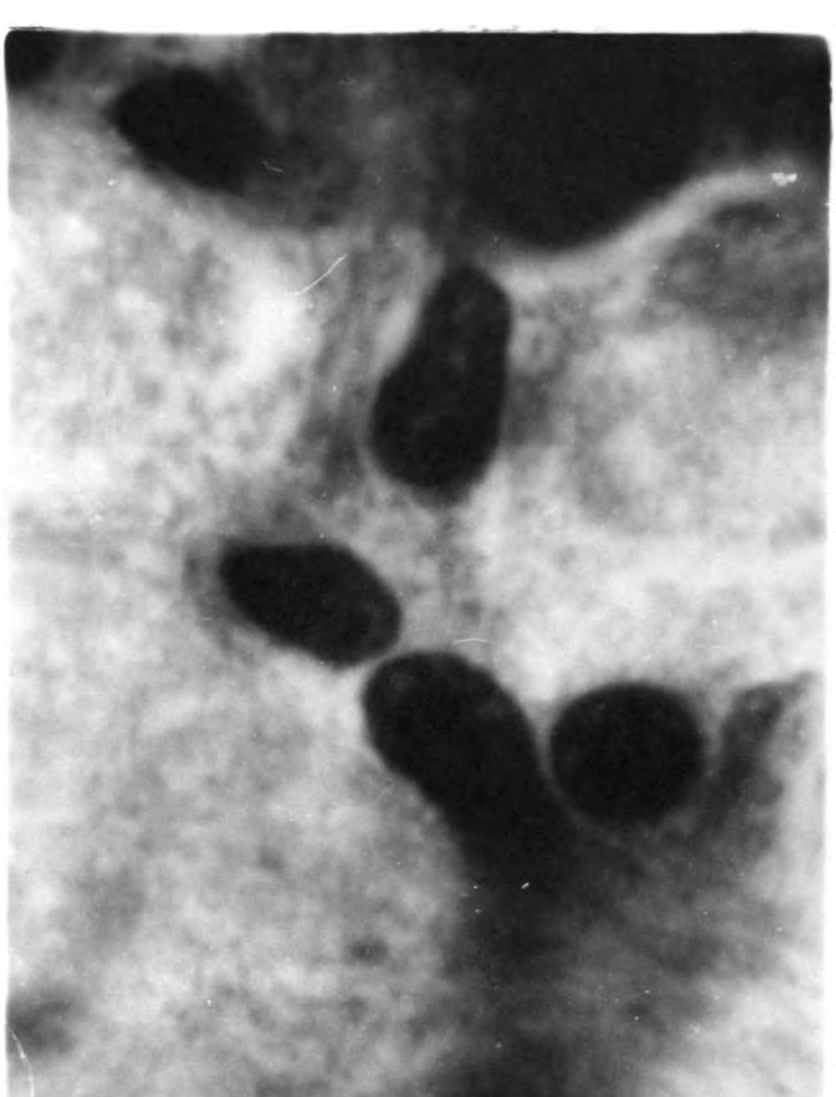

Fig. 2.--Median neurosecretory cells of the protocerebrum of female beetle in pre-diapause.
Stain--Paraldehyde fuchsin. Magnification 4050 X.

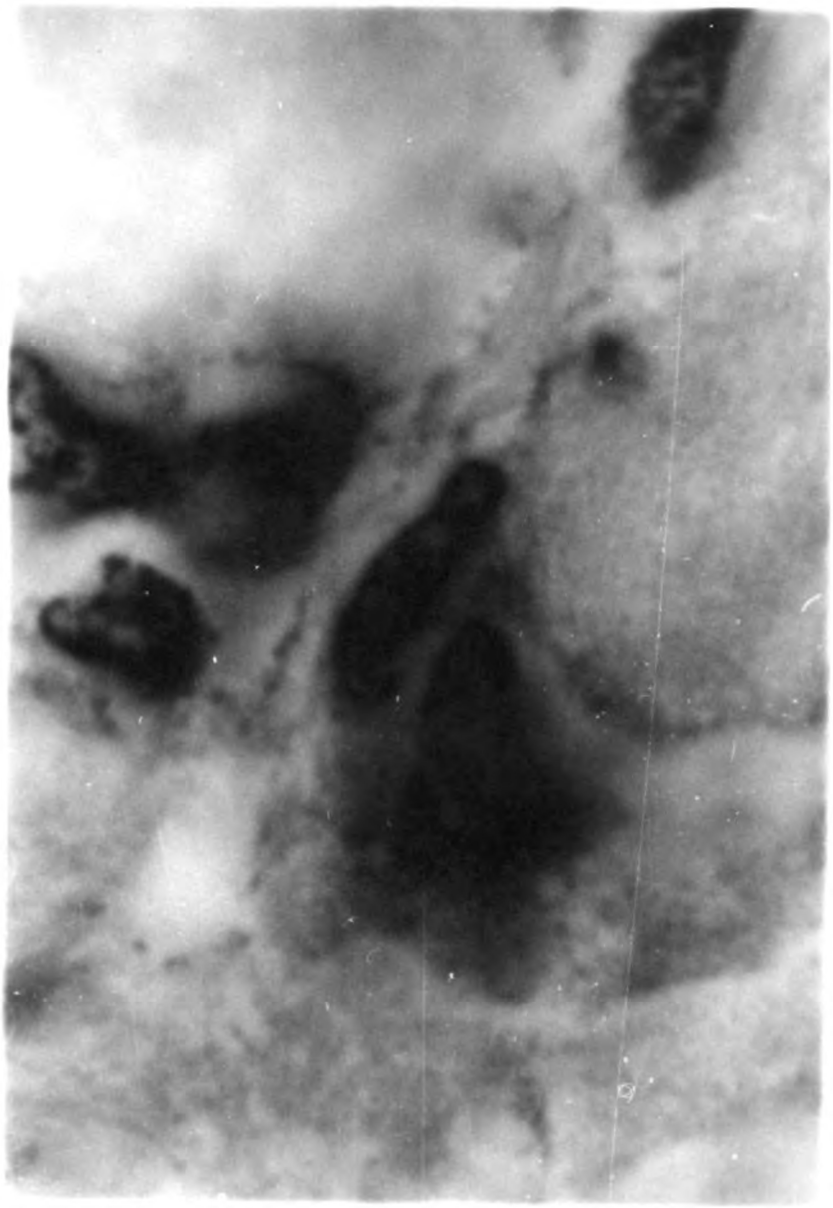

Fig. 3.--Median neurosecretory cells of the protocerebrum of female beetle in diapause.
Stain--Chrome haematoxylin/phloxine. Magnification 4050 X.

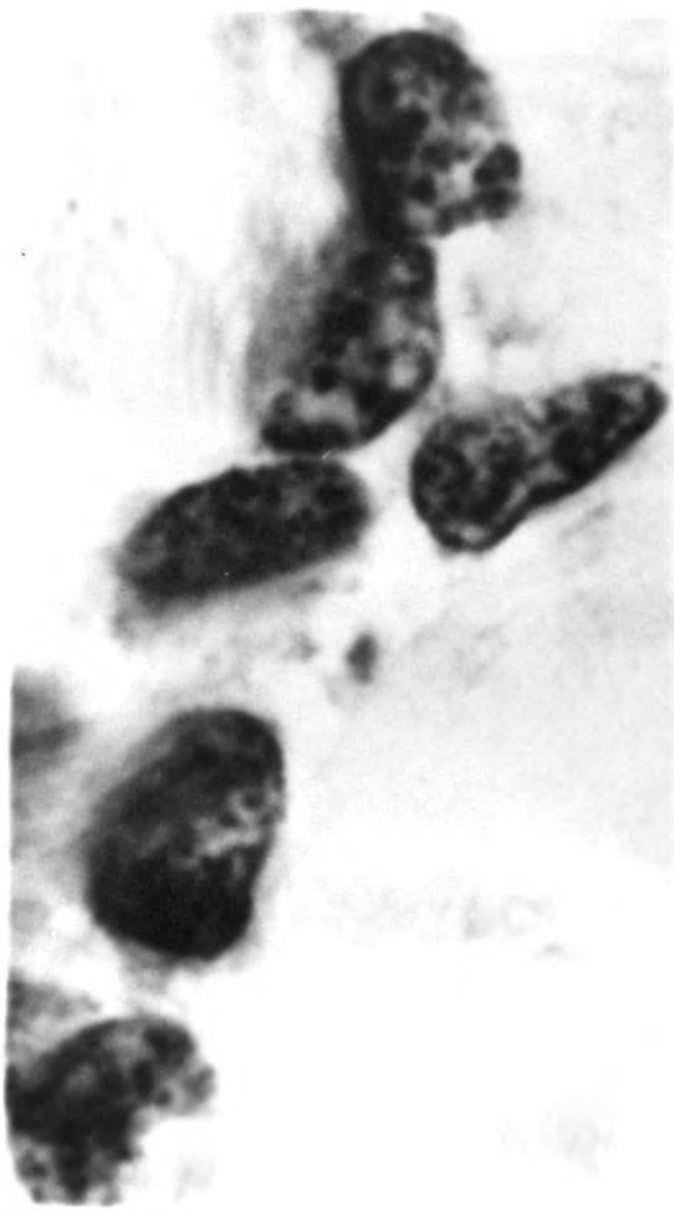

Fig. 4.--Median neurosecretory cells of the protocerebrum of female beetle in post-diapause.
Stain--Chrome haematoxylin/phloxine. Magnification 4050 X.

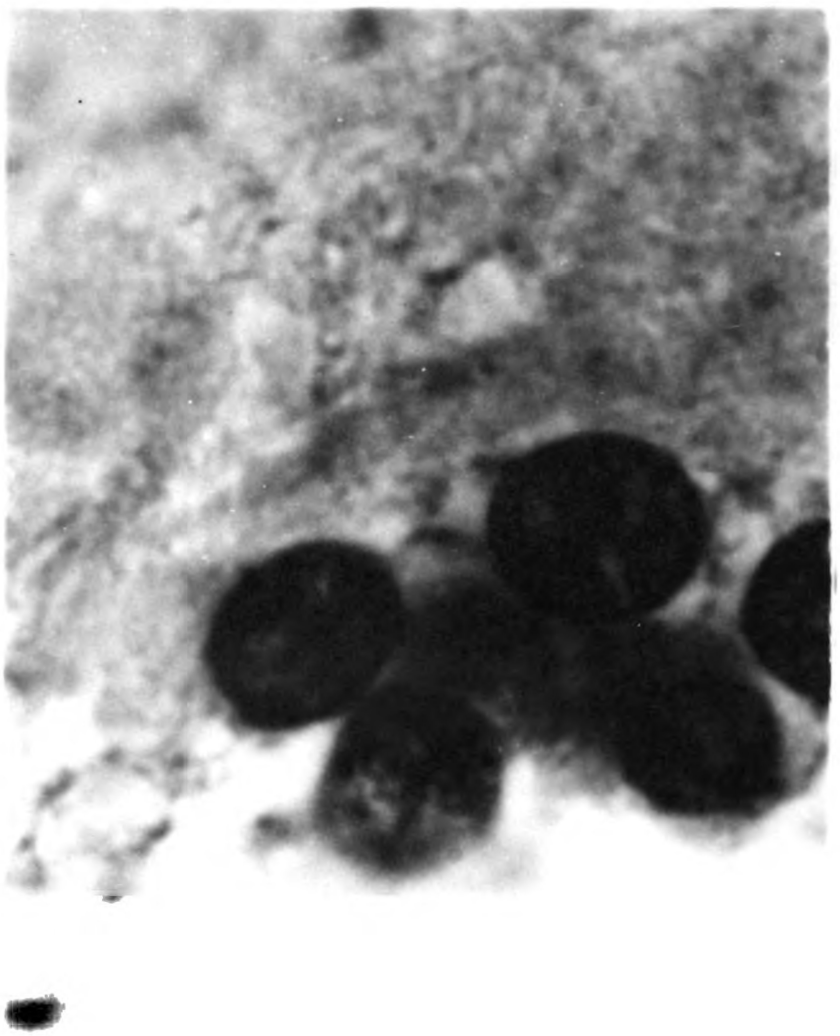

Fig. 5.--Median neurosecretory cells of the protocerebrum of O. melanopus in oviposition.
Stain--Chrome haematoxylin/phloxine. Magnification 4050 X.

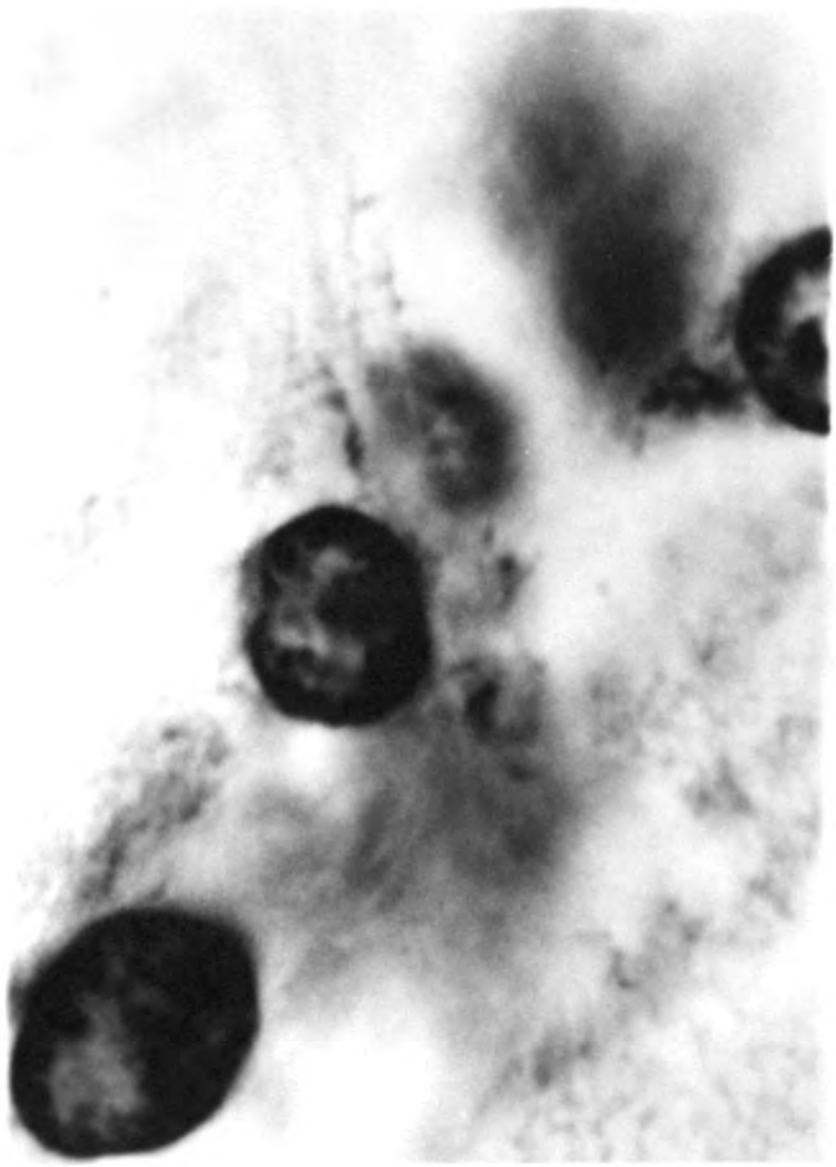

Fig. 6.--Median neurosecretory cells of the protocerebrum of O. melanopus in oviposition.
Stain--Chrome haematoxylin/phloxine. Magnification 4050 X.

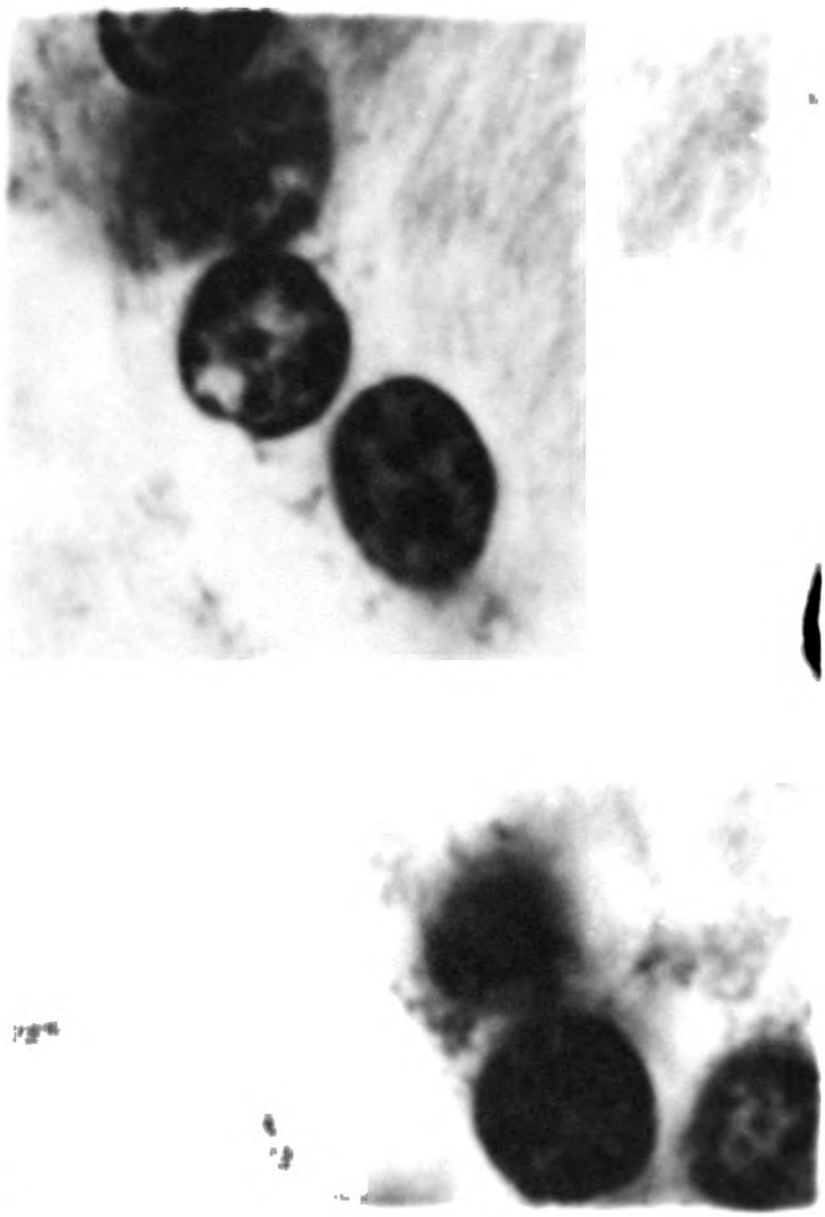

Fig. 7.--Median neurosecretory cells of the protocerebrum of O. melanopus in oviposition.
Stain--Chrome haematoxylin/phloxine. Magnification 4050 X.

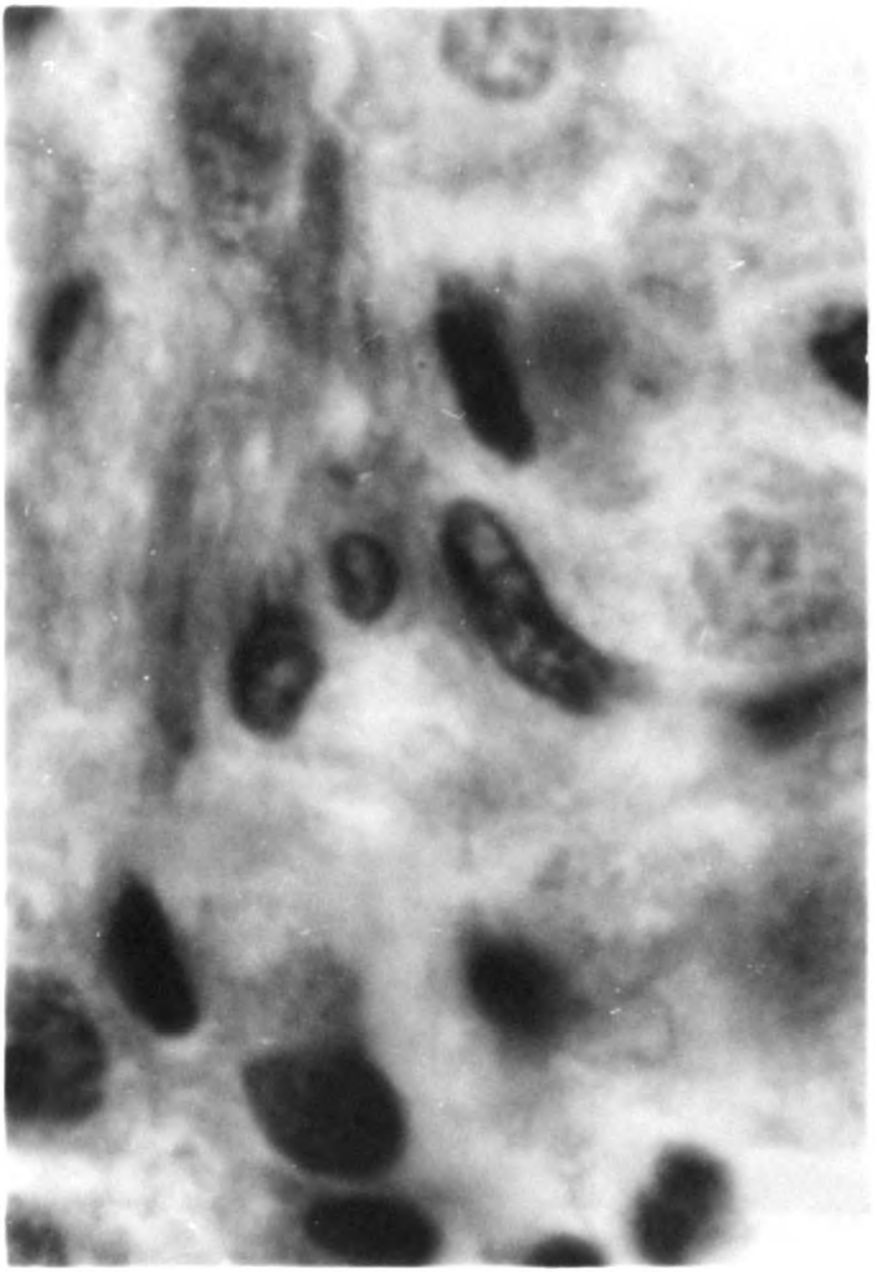

Fig. 8.--Section passing through the mid-portion of corpus cardiacum of female O. melanopus in pre-diapause showing two types of cells.
Stain--Paraldehyde fuchsin. Magnification 4050 X.

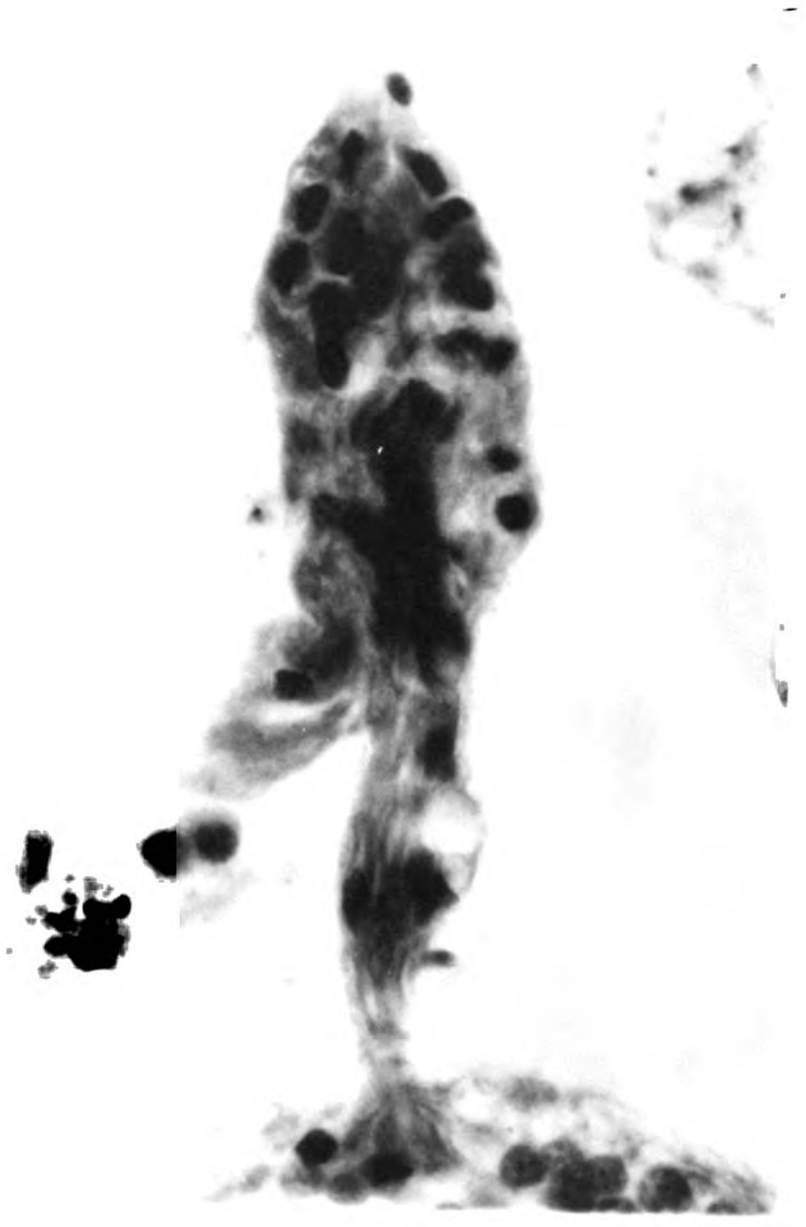

Fig. 9.--Section passing through the corpus cardiacum of female O. melanopus in diapause showing the presence of secretory cells in NCC I.
Stain--Paraldehyde fuchsin. Magnification 1100 X.

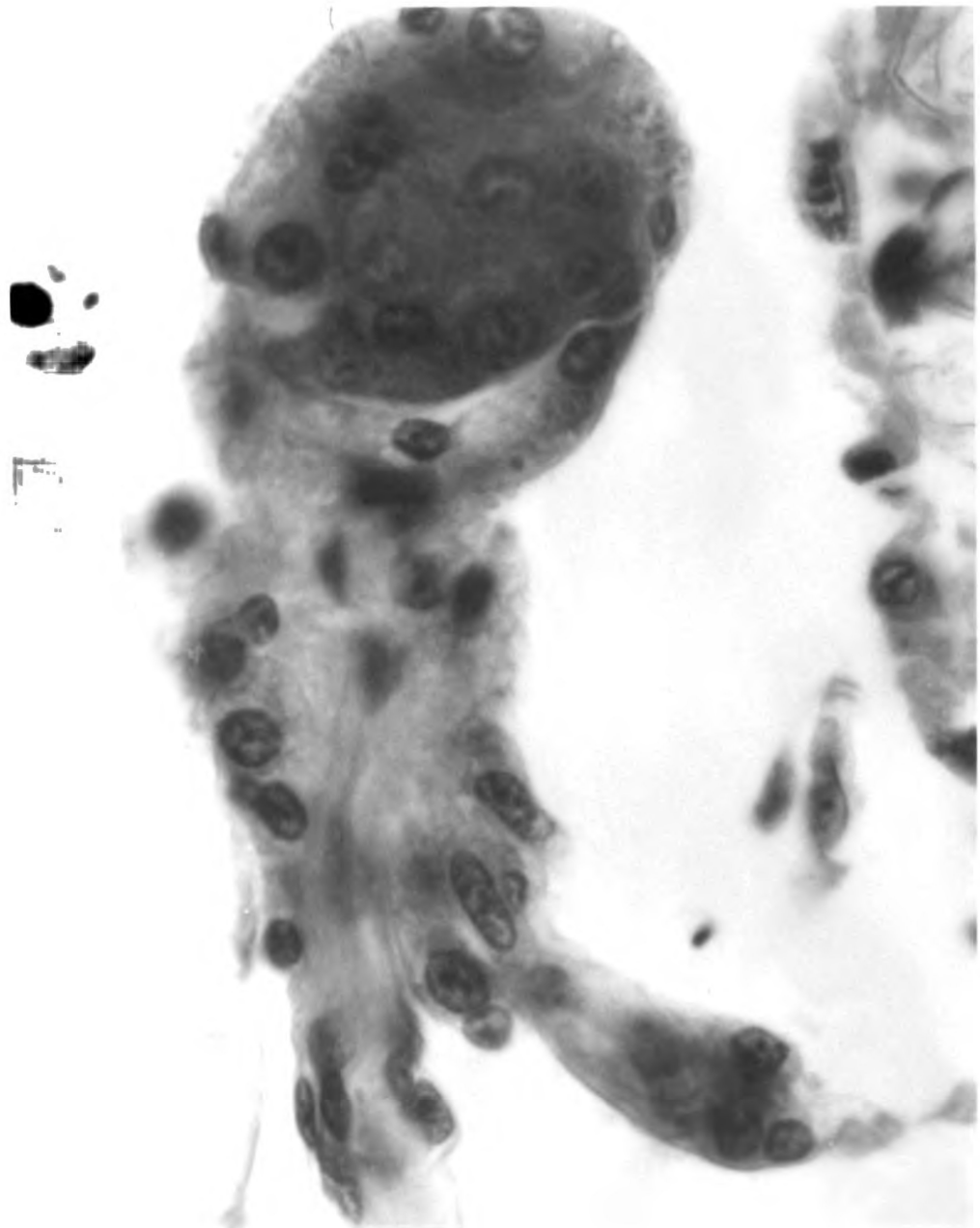

Fig. 10.--Section passing through the corpus cardiacum and corpus allatum of O. melanopus in oviposition showing the distribution of secretory cells.
Stain--Chrome haematoxylin/phloxine. Magnification 1600 X.

Fig. 11.--Section passing through the anterior portion of corpus cardiacum of O. melanopus in oviposition showing the presence of secretory cells.
Stain--Chrome haematoxylin/phloxine. Magnification 2700 X.

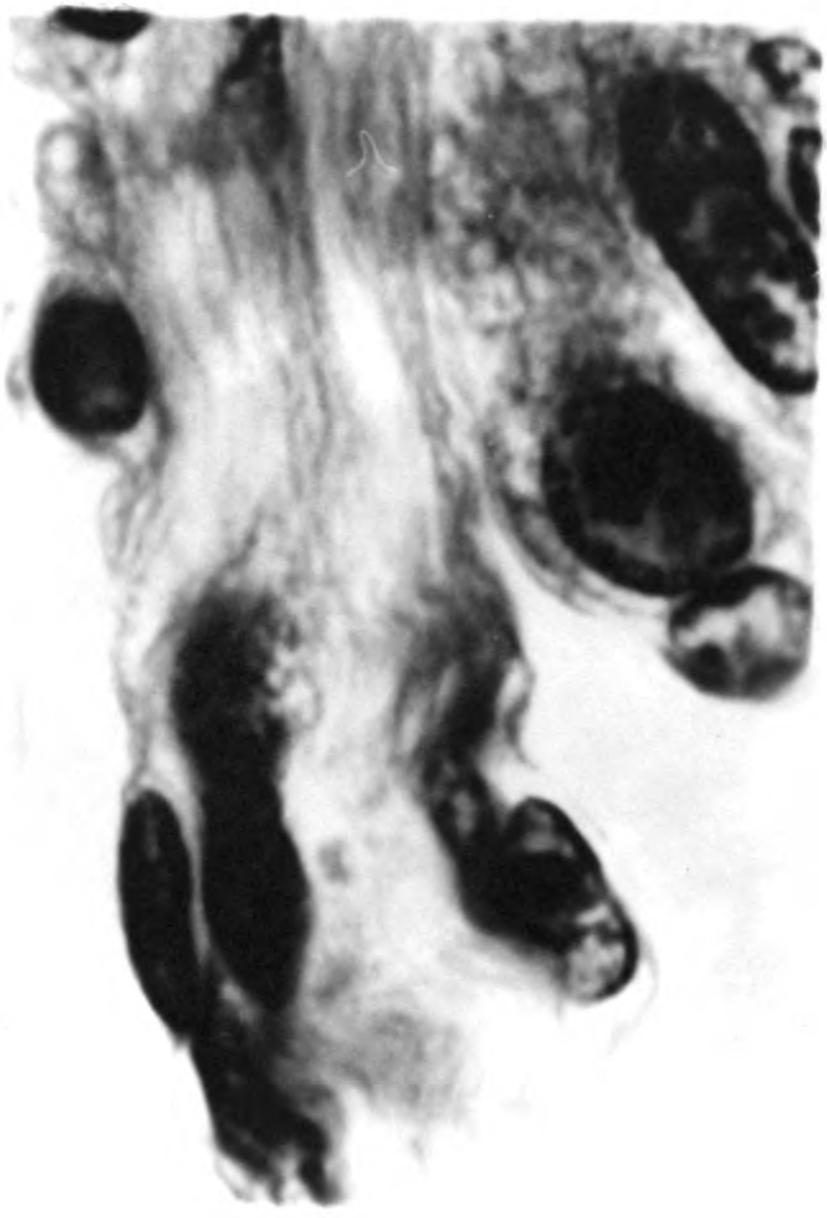

Fig. 12.--Section passing through the anterior portion of corpus cardiacum of O. melanopus in oviposition showing the presence of secretory cells.
Stain--Chrome haematoxylin/phloxine. Magnification 4050 X.

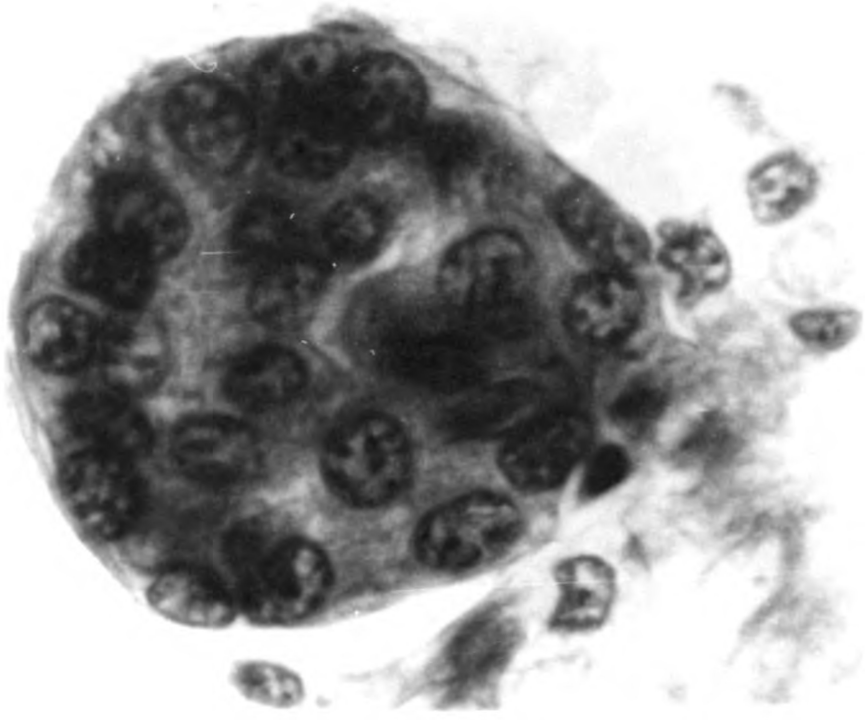

Fig. 13.--Section passing through the corpus allatum of male O. melanopus in pre-diapause showing homogeneous distribution of nuclei.
Stain--Paraldehyde fuchsin. Magnification 2700 X.

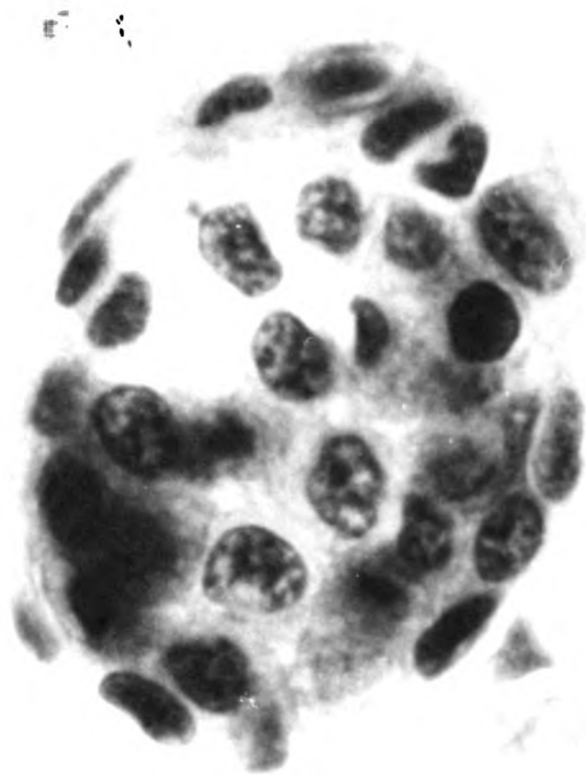

Fig. 14.--Section passing through the corpus allatum of female O. melanopus in pre-diapause.
Stain--Paraldehyde fuchsin. Magnification 2700 A.

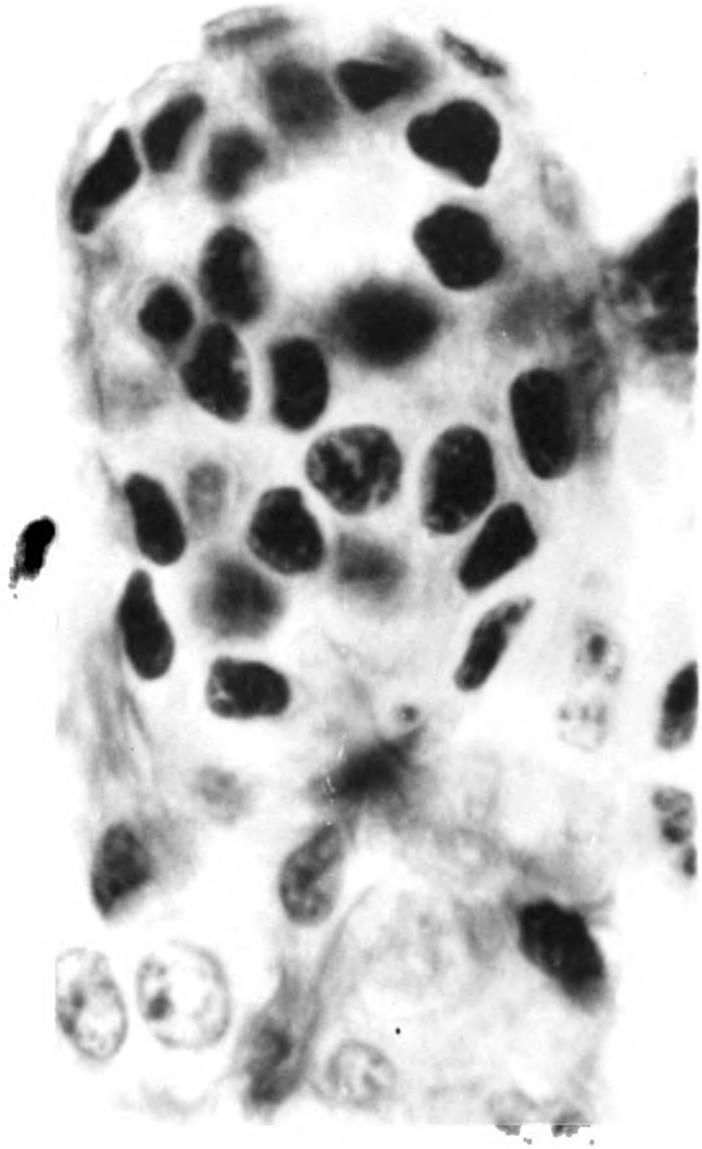

Fig. 15.--Section passing through the corpus allatum and posterior portion of corpus cardiacum of male O. melanopus in diapause showing some axons passing into the corpus allatum. Stain--Paraldehyde fuchsin. Magnification 2700X.

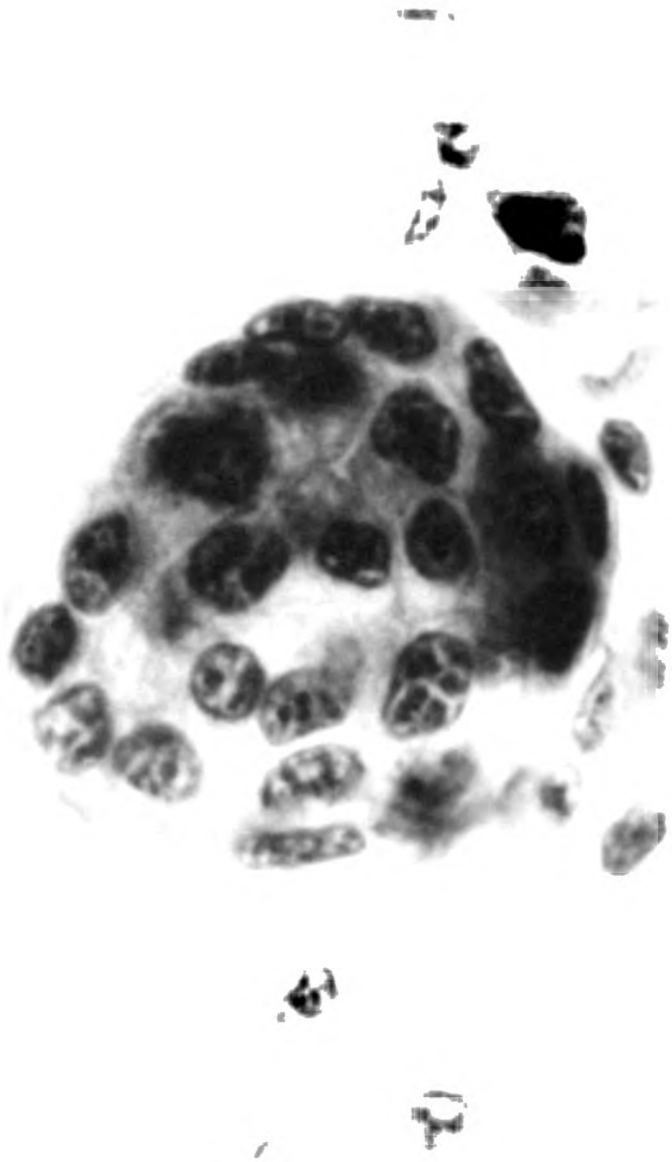

Fig. 16.--Section passing through the corpus allatum of female O. melanopus in diapause.
Stain--Paraldehyde fuchsin. Magnification 2700 X.

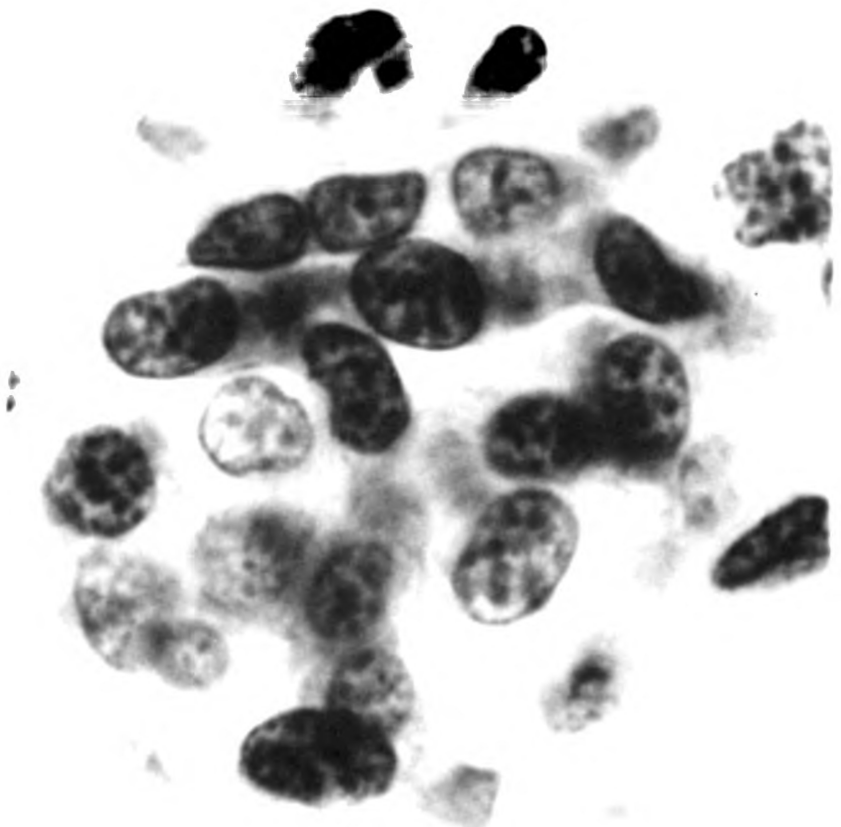

Fig. 17.--Section passing through the corpus allatum of female O. melanopus in post-diapause.
Stain--Chrome haematoxylin/phloxine. Magnification 2700 X.

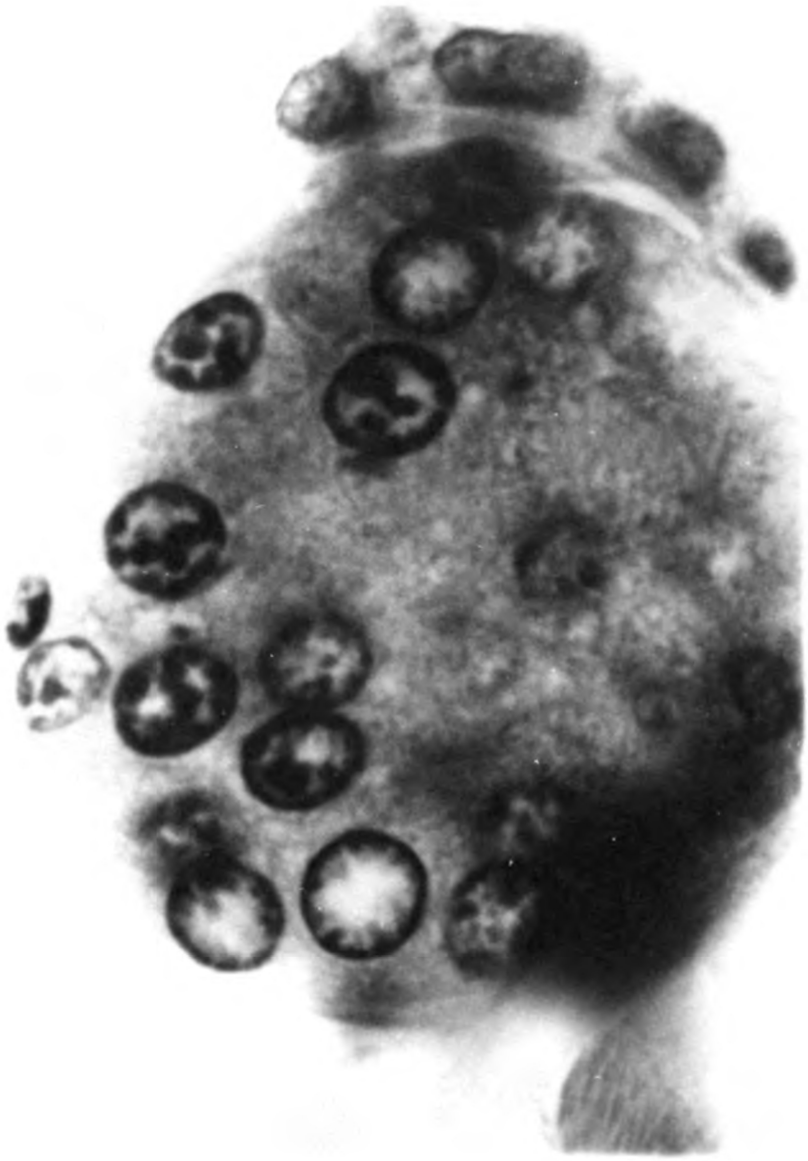

Fig. 18.--Section passing through the corpus allatum of O. melanopus in oviposition showing two types of cells. Stain--Alcian blue/phloxine. Magnification 2700 X.

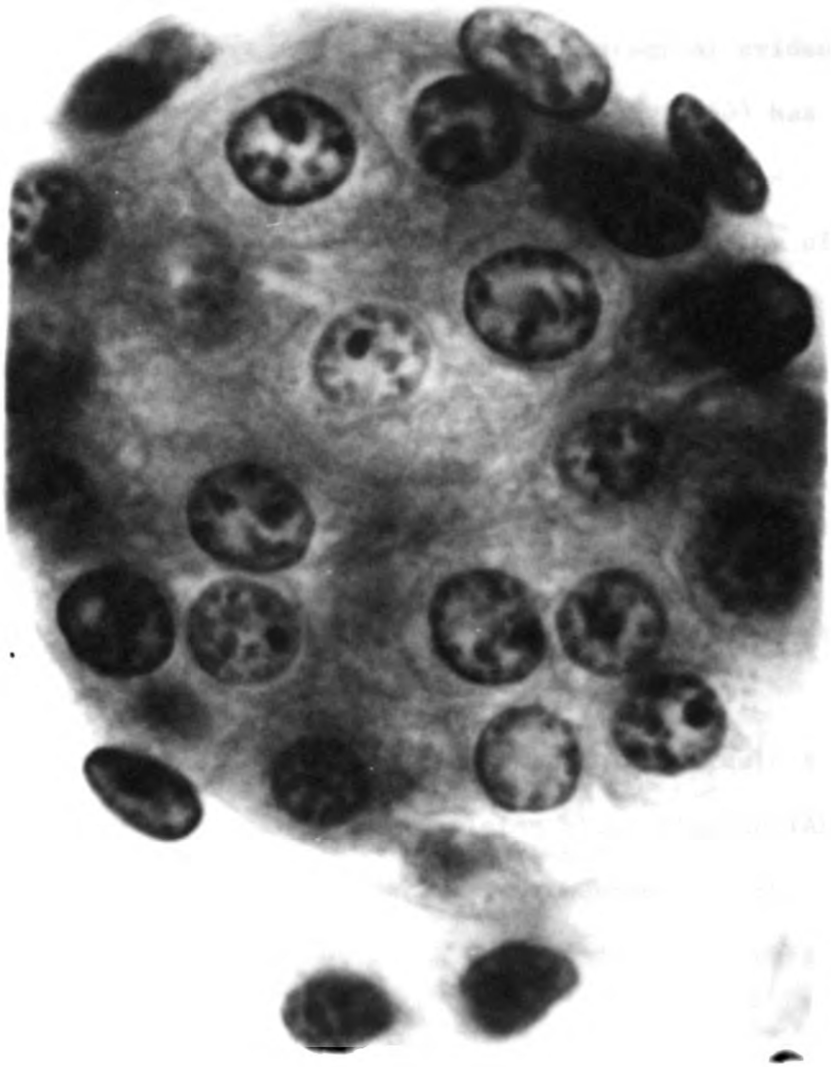

Fig. 19.--Section passing through the corpus allatum of O. melanopus in oviposition showing well defined nuclei distributed heterogeneously.
Stain--Alcian blue/phloxine. Magnification 2700 X.

DISCUSSION

It is evident from the results that Scharrer's (1954) definition of neurosecretory cells as nerve cells which show cytological evidence of secretory activity appears inadequate. Van den Kloot (1960) has described the neurosecretory cells which release hormones "neuro-endocrine" cells. In this study of neurosecretion the definition of Siew (1965) is followed. He included only those cells that, (1) showed cytological evidence of secretion, and (2) that were capable of discharging their secretions along their axons into the corpus cardiacum, and (3) showed cyclical morphometric changes during the different stages of the beetle's adult life.

The three staining techniques which have been most widely used for demonstrating neurosecretion are Gomori's (1941) chrome-haematoxylin/phloxine method (CHP), a modified Cameron and Steele's (1949) paraldehyde fuchsin method (PF), and alcian blue/phloxine (ABP). Using these staining techniques, two main types of neurosecretory cells (NSC), designated as A- and B-cells, have been described by a number of workers (Nayer, 1955, Siew, 1965).

The lateral groups of NSC of the protocerebral lobes in Oulema melanopus are comparable to those described in Iphita limbata (Nayar, 1955), Calliphora erythrocephala (Thomsen, 1952), Dermestes maculatus (Ladduwahetty, 1962), Galeruca tanaceti (Siew, 1965) and Aulacophora

faveicollis (Saini, 1966). However, the distribution of the cell types seems to vary in the few insects which have been studied. In Iphita limbata the lateral cells are phloxinophil and presumably the B-cell type (Nayar, 1955). In Dermestes maculatus this group contains five NSC, four of which are B-cells, where the fifth is a large C-cell. In Adelphocoris lineolatus (Ewen, 1962) only three B-cells are located in each lateral group. In G. tanaceti each group has two to three A cells and a variable number of D-cells depending on the physiological state of the insect. In Aulacophora foveicollis there are two B-cells in the lateral group. These cells color green with paraldehyde fuchsin and thus resemble B-cells, but with chrome-haematoxylin and alcian blue/phloxine they color blue and therefore are not similar to phloxinophic B-cells, hence D-cells (Siew, 1965). In O. melanopus lateral NSC contain both A and B-cell types. In this case B-cells stain yellowish green with paraldehyde fuchsin, red with alcian blue/ phloxine and A-cells stain orange with paraldehyde fuchsin and blue with alcian blue/phloxine, purple or blue black with chrome-haematoxylin, B-cells are representing phloxinophil cells stain red with ABP. The results obtained with O. melanopus can be compared with other insects as described above. It can also be concluded that the lateral groups of NSC in O. melanopus definitely contain A- and B-type of cells.

In the majority of insects which have been investigated, all the axons from the median neurosecretory cells end in the corpora cardiaca and corpora allata. A similar condition has also been observed in O. melanopus. Neurosecretory cells have been observed in the nervus corporis cardiaci I (NCC I) in all stages of the adult life

cycle. It can be concluded that in this beetle a certain amount of neurosecretory material is secreted in all stages of the adult. Ganagarajah (1965) has also reported the presence of neurosecretory material in Nebria brevicollis along the tracts of NCC I during breeding, but present in negligible amounts during pre-diapause. In O. melanopus all the neurosecretory cells show high activity during oviposition. Similar activity during oviposition has been observed in the beetle G. taneceti by Siew (1965). In the beetle, A. foveicollis only the median NSC show activity (Saini, 1966).

It was evident from the results that the activity of the neurosecretory cells of the brain in O. melanopus during oviposition was accompanied by an increase in activity of the corpora cardiaca and allata. There is also abundant evidence that the activity of the median neurosecretory cells in the brain is a primary requirement for the activity of the corpora allata (Thomsen, 1952; Highnam, 1962). During diapause the neurosecretory system functions, but probably at a low level, and is capable of controlling metabolism at the 'maintenance level'. The terminology 'maintenance level' has been described by Harvey (1962). This is contrary to what has been found in some cases of diapause, as in Hyalophora cecropia in which the neurosecretory cells are thought to cease their function at this phase (Williams, 1947). In Cephus cinetus there is evidence that the neuro-hormone from the NSC of the brain has to reach a certain level to be capable of activating the prothoracic glands (Church, 1955). The phase of ovarial development demands a high level of endocrine activity for the differentiation, development, and maturation of oöcytes.

The corpora cardiaca function normally as a depot and storage release center for neurosecretory material from the protocerebrum, as first pointed out by Scharrer (1951) in L. maderae. In contrast, the corpora cardiaca of O. melanopus never showed an accumulation of large amounts of neurosecretory material, which suggests that its storage function was slight. Gangarajah (1965) has reported in N. brevicollis that the corpora cardiaca did not serve as storage organs. Johansson (1958) also found in Oncopeltus fasciatus that the corpora allata do not act as storage organs, but that neurosecretory material is stored in the wall of the aorta in this insect.

Using the three staining techniques, two types of cells have been observed in the corpus allatum of O. melanopus. One type that stains purple with CHP and blue with ABP and light green with PF showed very distinct nuclei and cytoplasm, and the other stains red with CHP and ABP and yellowish orange with PF. One to three red globule like structures have also been observed within the nuclei in the purple and blue stained cells, besides the chromatin and nucleolus. Mendes (1948) has described four types of cells in the corpus allatum of Melanoplus differentialis. Siew (1965) has reported one type of cell in the corpus allatum of Galercica tanaceti Saini (1966) also describes one type of cell in the corpus allatum of A. foveicollis.

The role of the corpora allata in those insects which have been studied experimentally involves the control of yolk deposition in the female and the development of accessory glands in the male. It has been reported that both pre-diapause and diapausing O. melanopus showed arrested development or immaturity of the ovary (Kumararaj, 1964). It is evident from observation that the corpora allata remain

small during pre-diapause and diapause. During these periods the cells have crowded nuclei, scant cytoplasm, and lack of vacuoles, thus denoting a low level of activity. Similar type of results have also been reported in the beetle G. tansceti by Siew (1965). Ganagarajah (1965) has shown that the corpora allata in N. brevicollis (F.) remained small during pre-diapause and early diapause and also regressed in size towards the end of the breeding season. As a number of oöcytes normally develop from the germarium before yolk deposition begins, it would appear that in N. brevicollis, the corpora allata are necessary for the formation of oöcytes as well as for the deposition of yolk. In L. decemlineata, during diapause, the corpora allata are non-functioning (deWilde and Stegwee, 1958; deWilde and deBoer, 1961).

The corpus allatum of O. melanopus, in post-diapause, showed a steady increase in its volume and during the egg-laying period the gland increased to a maximum volume. There was a correspondingly marked increase in cytoplasmic content and the incidence of giant nuclei comparable to those described by DeLerma (1932), Palm (1947) and Scharrer and Harnack (1958). The peripheral cells of the corpus allatum increased in size far faster than those of the central region, and the gland assumed a characteristic heterogenous distribution of nuclei. Similar type of activity has been described to occur in beetle G. tanaceti (L.) (Siew, 1965). One of the most interesting things which appears in O. melanopus is that the level of the activity of the corpus allatum parallels the level of activity of the NSC of the brain. During oviposition the neuroendocrine system functions at a high level, probably to ensure the development of oöcytes and the process of oviposition.

Diapause is currently thought to be caused by a failure in the described humoral series of events (described before in Literature Review). Specifically, it is a failure of the brain to produce or to release the brain hormone. The data indicate that in O. melanopus there was a slight activity in the NSC in the pre-diapause period but this activity disappeared during diapause. During oviposition a renewal of activity in the NSC of the brain occurred. This activity was accompanied by an increase in the activity of the corpus allatum. There was evidence that the activity of the median NSC in the brain was a primary requirement for the activity of the corpora allata (Thomsen, 1952 and Highnam, 1962). In this beetle the corpora allata remained inactive during diapause and showed maximum activity during the egg laying period. It is possible that diapause in O. melanopus was a result of an inactive corpus allatum which is in agreement with that which occurs in the beetle L. decemlineata as described by deWilde and his co-workers (1953).

SUMMARY

The median, lateral and anterio-ventral groups of neurosecretory cells were observed in the protocerebrum of Oulema melanopus. The neurosecretory cells showed cyclical morphometric changes at the different stages of the beetle's adult life. During oviposition the neurosecretory cells of protocerebrum showed maximal activity and during diapause showed the least activity.

Three staining techniques were used in histophysiological study of neurosecretion. Using these three staining techniques, two types of neurosecretory cells (A and B) were observed. The B-cell types were phloxinophil, staining red with chrome haematoxalin/phyloxine and alcin blue/phyloxine. Similar type of cells have been observed in other beetles.

Secretory cells were present in nervus corporus cardiaci I and II. Some of the axons of nervus corporus cardiaci I and II which innervate the corpus cardiacum were also shown to innervate the corpus allatum.

The increase in the size of the corpus cardiacum was attributed mainly to increase in the cytoplasmic content of the gland. The secretory 'chromophile cells' enlarged, but there was also an increase in the nuclear volumes at oviposition.

Two types of cell were observed in the corpus allatum. During the inactive phase the corpus allatum was characterized by cells with

low cytoplasmic content and closely packed nuclei. During pre-diapause and diapause phase the volume of the gland was small and at the termination of diapause the gland increased steadily in volume. During the egg-laying period the gland increased to a maximum volume. The peripheral cells of the corpus allatum were generally larger than those of the middle region of the gland. No mitotic activity was observed at any stage.

The different stages of the adult beetle differed distinctly in their respiratory rate. Diapause beetles showed the lowest oxygen uptake while pre-diapause beetles the greatest amount of oxygen uptake.

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