ABSTRACT

M.Sc. Gerald R. Buzzell Parasitology

THE RETROCEREBRAL SYSTEM
OF THE ADULT SHEEP KED

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Suggested short title:

THE RETROCEREBRAL SYSTEM OF THE ADULT SHEEP KED

Buzzell

THE RETROCEREBRAL SYSTEM OF THE ADULT SHEEP KED

by

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A thesis presented to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of Master of Science

Institute of Parasitology
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PREFACE

The research described in this thesis was carried out at the Institute of Parasitology of Macdonald College of McGill University, in Ste. Anne de Bellevue, Quebec, between May, 1967 and August, 1968. Financial assistance from McGill University in the Solvay Fellowship and from the Institute of Parasitology in a studentship are gratefully acknowledged.

Techniques and observations not strictly related to the theme of this thesis are placed in appendices at the end of the thesis. Drawings, photographs, tables, and graphs are placed at the end of the chapter or appendix in which they are first cited. A uniform system of labelling figures is used; this is described at the end of the list of figures.

Professor K. G. Davey, Director of the Institute of Parasitology, supervised this project. His advice and encouragement are greatly appreciated.

All members of the Institute of Parasitology have given invaluable encouragement to me during the course

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LIST OF FIGURES

A uniform system of labelling figures has been used. The following are the symbols used and their meanings. Those which are not mentioned in the text are defined; others are merely named and the reader should refer to the text for the definition.

a. aorta.
b. brain.
c. cuticle.
ca. corpus allatum.

cc.	corpus cardiacum.
cc-hg	fused corpus cardiacum and hypocerebral ganglion.
cc-pp	posterior prolongation of the corpus cardiacum in Tipula sp.
cl.	cortical layer. In the brain (and other ganglia), the peripheral layer of nerve cells.
crn.	cardiacum-recurrent nerve of Calliphora erythrocephala.
cs.	strand of cuticle passing into the structure around the ventral nerve cord in the neck region (Appendix E).
ct.	connective tissue.
dm.	the "dorsal mass" of Aedes aegypti.
fb.	cells of the fat body.
fg.	frontal ganglion.
gc.	the "gland complex" of Aedes aegypti.
hg.	hypocerebral ganglion.
ic.	intrinsic secretory cells of the corpus cardiacum.
lnc.	lateral neurosecretory cells.
m.	muscle.
mnc.	medial neurosecretory cells.
n.	nerve fibres in the corpus cardiacum.
nca.	nervi corporis allati.
ncc.	nervi corporis cardiaci.
ncc I.	nervi corporis cardiaci I.
ncc II.	nervi corporis cardiaci II.
ndm.	nerve to the "dorsal mass" of Aedes aegypti.
ngc.	nerve to the "gland complex" of Aedes aegypti.
nl.	neurilemma. A layer of connective tissue surrounding nervous structures.
np.	neuropile. An area of nerve fibres forming the "core" of any ganglion.
nt I.	nerve tract leading from the medial neurosecretory cells to the ncc I.
nt II.	nerve tract leading from the lateral neurosecretory cells to the ncc II.
o.	oesophagus.
oc.	oesophageal channel.
on.	oesophageal nerve. A nerve that travels between the hypocerebral ganglion to the stomatogastric nervous system.
p.	proventriculus. The region of the alimentary canal immediately posterior to the oesophagus.
pi.	pars intercerebralis.
rn.	recurrent nerve.
sc.	"small cells" of the corpus cardiacum.
sd.	salivary duct. Duct that leads from the salivary glands to the oesophagus.

sg.	suboesophageal ganglion.
t.	trachea.
tg.	thoracic gland.
tgl.	thoracic ganglion.
tn.	tendon.
vn.	ventral nerve cord.
x.	the structure around the ventral nerve cord in the neck region.

CHAPTER I

THE PROBLEM

The control of destructive insects is usually chemical, by the spreading of poisons (insecticides) on their habitats. As has been discussed by many people, among the most eloquent of whom is Rachel Carson (1962), this is undesirable. Insecticides lack specificity; they kill not only insect pests but also harmless and beneficial insects and they are harmful to other animals, including man. In finding an alternative to insecticides, the idea arose of turning the insect's physiology against itself.

In 1958, a large number of sterile male screw-worm flies were released into the southeastern United States; these, by competing with normal males, helped cause the eradication of the fly from this area (Knipling, 1960). This pointed dramatically to the vulnerability of the insect's reproductive system and to the possibility of controlling insects by sterilization. Sterility was produced in the screw-worm fly by irradiation (Knipling, 1960). An alternative means of

sterilizing insects has been by the use of chaemosterilants (Borkovec, 1966).

Williams (1967) pointed out the possibility of using insect hormones as pesticides. The so-called "paper factor," an analogue of the juvenile hormone, has been found to prevent adult development in some members of the family Pyrrhocoridae (Hemiptera), but not in other insects. Although the target of the paper factor is development in general, the net result is sterility, since these insects do not reach sexual maturity. A great advantage is the fact that the paper factor is very specific.

Much research has been done in the last thirty years on the endocrine control of insect reproduction. This approach may, in the future, be fruitful for insect control. One can visualize hormone pesticides acting directly on the reproductive processes rather than indirectly by their effects on general developmental processes. This would have the added advantage of allowing sterilized adults to compete with the normal adults of the species, with the result that complete sterilization of the population might not be necessary for control.

Among the more medically important insect pests are the tsetse flies, Glossina sp. These act as vectors of the haemoflagellates Trypanosoma gambiense and T. rhodesiense, the causal agents of African sleeping sickness. The control of the tsetse flies and the consequent eradication of this disease would improve health conditions over a large part of tropical Africa. Perhaps a knowledge of the endocrine control of reproduction in the tsetse flies would lead to the eventual elimination of this pest. Unfortunately, Glossina is a tropical fly and has proved to be quite difficult to rear in laboratories (Langley, 1967a).

The sheep ked, Melophagus ovinus, a dipteran ectoparasitic on sheep, may be an acceptable substitute for the tsetse fly in studies on endocrines and reproduction. These two dipterans have remarkably similar reproductive cycles. Both are viviparous and nourish the larva, which develops in the female's uterus, from secretions of a "milk-gland." At parturition, both types of flies ovulate and development of another larva starts. Soon after parturition, larvae of these two flies pupate. These similarities are remarkable examples of convergent evolution.

Melophagus has the added advantage of being native to Canada (as well as to nearly every other area where sheep are kept) and easy to raise. During the autumn and early spring, sheep harbour large numbers of the parasite. Use of the ked has the disadvantage that the parasite is unable to survive off sheep for longer than a few days and so is difficult to observe continuously. The advantages of using the ked as a model for studying the endocrine control of reproduction in the tsetse fly far outweigh this disadvantage.

For these reasons, I studied the endocrine control of reproduction in the ked. It is hoped that my results may aid researchers working to control the tsetse fly as well as to add to the general knowledge of insect endocrine systems. In addition, I hope readers will come away, as I did, with the impression that Melophagus is an unusual and interesting insect, and that more research will be done in the future on this fascinating and little-known fly.

CHAPTER II

MELOPHAGUS OVINUS: THE EXPERIMENTAL ANIMAL

The sheep ked, *Melophagus ovinus* (Fig. 1), is a fly ectoparasitic on sheep. According to the scheme of classification given in Imms (1957), it belongs to the Order Diptera, the Suborder Cyclorrhapha, the Section Pupipara, and the Family Hippoboscidae.

The most common host of the ked is the domestic sheep, *Ovis aris*. It has been reported from wild sheep. In fact, Ferris and Cole (1922) made a distinction between *Melophagus ovinus ovinus* of domestic sheep and *M. o. montanus* of wild sheep; Bequaert (1942), considers this distinction to be trivial. Keds are sometimes found on goats, especially on those kept close to sheep; whether they feed and reproduce on them is unknown (Bequaert, 1942). They have been found occasionally on cattle, horses, dogs, wolves, coyotes, and humans. These hosts presumably obtained keds from sheep and proved to be dead-ends; the parasites soon died (Bequaert, 1942). Rodhain and Brutsaert (1935) induced a few keds to feed on guinea pigs; this has

not, to my knowledge, been repeated. For all practical purposes, Melophagus ovinus is completely host specific, that host being the domestic sheep, Ovis aris.

A. Life Cycle

The entire life cycle of the ked is spent on sheep; dispersal is by contact. Pupae are attached to wool fibres at variable distances from the skin; these distances gradually increase as the wool grows (Graham and Taylor, 1941).

Newly emerged adult keds first feed 24 to 36 hours after emergence and continue to feed at approximately 36-hour intervals thereafter (Nelson, 1955). Keds can survive off the sheep without feeding for only four or five days (Graham and Taylor, 1941).

Maturation and copulation by male keds take place around ten days after emergence (Graham and Taylor, 1941; Evans, 1950). Female keds mate 16 to 24 hours after emergence, but do not ovulate until they are six or seven days old (Graham and Taylor, 1941; Evans, 1946, 1950). Spermatozoa are stored in the female's receptaculum seminalis (Pratt, 1899).

One mating is sufficient to last for the ked's lifetime, although matings occur frequently when possible (Graham and Taylor, 1941; Evans, 1946, 1950). This is of probable significance during the summer and autumn when sheep become resistant to keds and few of the parasites survive (Nelson and Qually, 1958). Evans (1950) suggested that this fact might be important for the spreading of infestations to ked-free sheep. One fertilized female ked, transferred to one sheep by contact with another, should be sufficient to produce a large population of the parasite on that sheep. However, Nelson (1958) found that, at least on lambs, only newly emerged virgin keds are infective, an observation that, if true, seriously limits the importance of Evans' suggestion.

Keds (and all Pupipara) are viviparous. One larva at a time develops in the female's uterus and, unlike the case of most other viviparous insects (Davey, 1967a; Wigglesworth, 1965), is nourished from secretions of her "milk gland" (Pratt, 1899). This type of viviparity is very similar to that of the tsetse fly, Glossina (see above, Chapter I).

Bequaert (1942) cites Berlese (1899) as being of

the opinion that larval keds do not feed on the secretions of the milk glands but rather on excess spermatozoa and male accessory secretions. Bequaert feels that the true situation is a combination of these two views.

An unknown number of larval stages is spent in the uterus (Day, 1943b); the gestation period is six to eight days (Graham and Taylor, 1941; Evans, 1946, 1950). Parturition occurs just previous to pupation; one case of intra-uterine pupation has been reported (Root, 1921) but this observation has never, to my knowledge, been repeated. The pupal stage lasts 19 to 26 days, depending on the temperature (Graham and Taylor, 1941; Evans, 1950).

Adult female keds, which live approximately 100 days, survive about 20 days longer than do males. They produce, on the average, 10 to 15 offspring each (Graham and Taylor, 1941).

B. Economic Importance

Keds are hosts to a number of parasites and transmit some of these to sheep. Most of these parasites are not pathological to the sheep.

The sheep trypanosome, Trypanosoma melophagium, is transmitted to sheep by keds. Originally, in fact, it was considered to be purely a ked parasite (Crithidia melophagium) because it could not be isolated from sheep's blood. Hoare (1923) succeeded in proving its existence there by culture techniques and demonstrated that its transmission is contaminative, by the eating of infected keds by the sheep.

T. melophagium is harmless to sheep; however, there is some evidence that it may cause ked mortality by blocking the posterior midgut of the insect (Nelson, 1956, 1957).

Keds have been shown experimentally to be susceptible to infection by Trypanosoma cruzi and T. lewisi (Rodhain and Brutsaert, 1935). This, because of the normal host range of these trypanosomes and the host specificity of keds, never happens in nature.

Another experimental infection of Melophagus could, conceivably, have quite serious consequences. This is that of the virus causing blue-tongue disease. This virus, usually spread by Culicoides, has been found experimentally to be potentially spread by the ked (Gray and Bannister, 1961; Luedke et al., 1965).

In nature, keds could become quite important vectors of the disease in sheep. Nelson's (1958) observation that only newly emerged keds infect lambs (see above, p. 7), if one assumes that trans-ovarian transmission of the virus does not take place, probably explains why this has not happened.

Melophagus is also the host of a harmless rickettsia, Rickettsia melophagi (Bequaert, 1942).

Arguments as to whether Melophagus is harmful to sheep have been going on for years. Imes (1932) sums up the case for the prosecution quite well as follows: "The irritation caused by the ticks [keds] makes the sheep restless so that they do not feed well, and in consequence, they do not grow and fatten as rapidly as when free from ticks [keds]. Thus a loss is caused by shrinkage in weight and a general unthrifty condition of infested flocks, with a consequent lowering of the vitality and a reduction in the resisting power of the animals."

On the other hand, Pfadt et al. (1953) and Whiting et al. (1954), in testing the hypothesis that the presence of keds causes weight loss in feeder lambs, found no significant differences between the experimental

and the control observations.

A recent report by Nelson and Slen (1968) reveals that ked-free lambs gain more weight and ked-free ewes produce more wool than infested lambs and ewes respectively. These differences, thay maintain, are apparent only after sheep have become resistant to keds.

My personal observations reveal that sheep with keds are irritable, scratch themselves, and rub against objects. The resulting scruffiness may detract from the market value of the wool. So, economically and from a point of view of the sheep's comfort, keds are unwelcome pests.

The most common methods of combatting keds are by sheep dips (Imes, 1932; Treeby, 1967) and by insecticide sprays (Matthysse, 1967). More than one application is usually necessary as ked pupae are resistant to most of the common procedures. The expenditure of time, effort, and money in attempting to control keds, by themselves, makes the parasite of considerable economic importance.

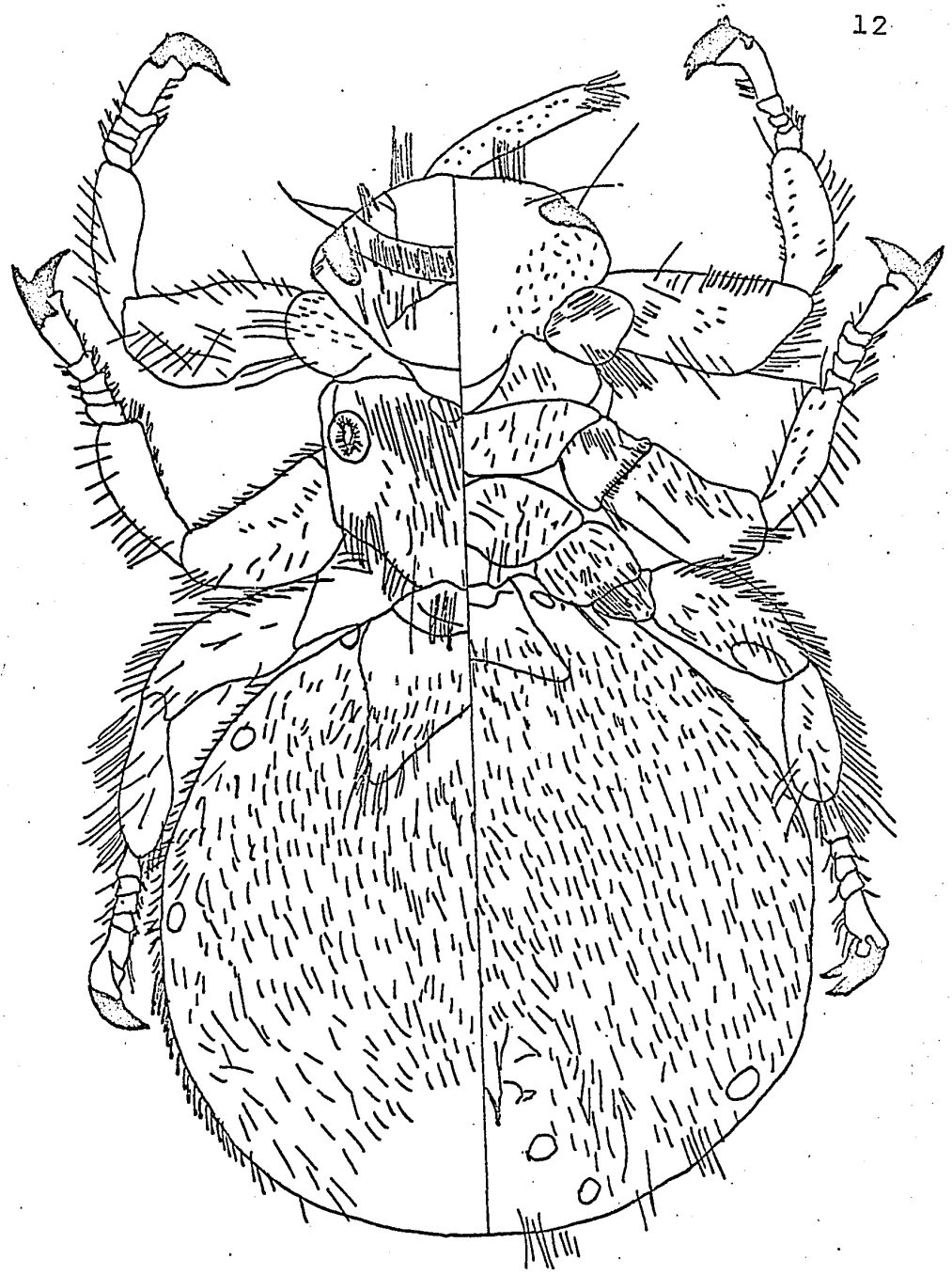


Figure 1. The sheep ked, Melophagus ovinus. Dorsal view (left) and ventral view (right) of a male. (After Ferris and Cole, 1922).

CHAPTER III

THE INSECT RETROCEREBRAL SYSTEM

The insects comprise an extremely large and diverse group of animals. It follows that there is a correspondingly large and diverse assortment of anatomical arrangements of the various organ systems; the endocrine system is a case in point. Although there are certain unifying characteristics, there are so many different arrangements of the organs and their nerve supplies that the mind recoils at the thought of describing them all. Cazal (1948) has described this system in a few representatives of the major insect groups and his work, though of necessity quite superficial, remains the classic paper on this subject. Recent reviews on insect hormones, concerned mainly with studies of their physiology, have been written by Novák (1966), Ralph (1967), and Highnam (1967).

The insect endocrine system is very closely associated with the central nervous system and the retrocerebral nervous system. In fact, the term "retrocerebral system" is often used as a synonym for

"endocrine system" as, for instance, in the title of this thesis. It should be noted that, strictly speaking, these two terms are not synonymous. Insects have endocrine components with no relation at all to the retrocerebral nervous system. These will not be considered at all in this thesis.

In this chapter, a brief description will be given of the retrocerebral system in a "generalized" insect. Then descriptions will be given of the morphology of several representative dipteran retrocerebral systems. The role of the retrocerebral system in the control of insect reproduction will then be briefly discussed.

A. The Generalized Insect Retrocerebral System

The insect retrocerebral system has four main endocrine components: neurosecretory cells in the brain, the corpora cardiaca, the corpora allata, and the thoracic gland or its homologue (Fig. 2).

Neurosecretory cells are nerve cells that manufacture and secrete hormones (for recent reviews, see Bern, 1966; Knowles and Bern, 1966; Scharrer, 1967).

They are characterized on both morphological and physiological grounds. Morphologically there are three criteria for distinguishing them from ordinary nerve cells. In vivo, they often have a blue appearance (Tyndall blue) (E. Thomsen, 1954; Novák, 1966). They stain in characteristic ways with such stains as paraldehyde fuchsin (PAF), chrome-alum-haematoxylin-phloxine (CAHP), performic acid alcian blue (PFAAB) (Scharrer, 1967), and azan (de Bessé, 1967). In electron micrographs, they contain characteristic membrane-bound vesicles (Bern, 1966; Scharrer, 1967). Physiologically, they are characterized by detectable cycles of manufacture and secretion that parallel their physiological effect. They may be further characterized on physiological grounds by the results of extirpation and implantation experiments (Novák, 1966).

Neurosecretory cells have been found in most groups of multicellular animals, from a somewhat dubious report of them in sponges (Lentz, 1966) to the vertebrates and man. (Lentz' report, I consider to be questionable because nerve cells have not yet been proven to exist in sponges. It is all too seldom emphasized that neurosecretory cells must be nerve cells.)

In insects, neurosecretory cells are concentrated in the mid-dorsal region of the protocerebrum, the pars intercerebralis; however, they are also found elsewhere in the supraoesophageal ganglion, in the suboesophageal ganglion (see, for instance, M. Thomsen, 1965), and in the thoracic ganglia (de Bessé, 1967). In the pars intercerebralis are two main groups of neurosecretory cells, the medial and the lateral (Fig. 2). These may be differentiated by their staining reactions (see below, p. 70) and by their observed cycles of activity (Novák, 1966).

The medial neurosecretory cells have been found to secrete several hormones. The earliest discovered was the so-called "brain hormone" which is secreted via the corpus cardiacum and stimulates the thoracic glands to secrete ecdysone which causes moulting (Wigglesworth, 1954, 1965). A gonadotropic hormone (E. Thomsen, 1952) and one that stimulates gut protease synthesis (Thomsen and Møller, 1963) have also been found; as is discussed below (pp. 29-31), these latter two effects may be caused by the same hormone. A myotropic hormone has been found in Rhodnius prolixus (Hemiptera) that is at least partially responsible for an increase in the rate of oviposition that accompanies mating (Davey, 1967b).

The axons of the medial and the lateral neurosecretory cells transport neurosecretory material to the corpora cardiaca. Paired nervi corporis cardiaci I (NCC I) and NCC II are formed from these axons, those of the medial neurosecretory cells forming the NCC I and those of the lateral ones forming the NCC II (Fig. 2). Other groups of neurosecretory cells may give rise to other NCC's that pass to the corpora cardiaca; there are four pairs in several Dictyoptera, Isoptera, Orthoptera, and Heteroptera (Brousse-Gaury, 1967). On the other hand, the NCC I and NCC II may fuse into a single pair of nerves, the NCC. This is the case with most of the Diptera (Cazal, 1948).

The NCC pass posteriorly to the corpora cardiaca. These paired organs, often fused into one (Fig. 2), have two main components. One consists of the endings of the cerebral neurosecretory cells; here the secretion is stored and released when needed. (This type of structure is called a "neurohaemal organ.") Intrinsic secretory cells comprise the other component. The corpora cardiaca are often closely associated with the hypocerebral ganglion and/or the aortal wall. In fact, they may fuse with one or both of these structures.

The close association of the corpora cardiaca with the aorta is seen from the fact that, in Dysdercus koenigii (Hemiptera), the NCC I do not transport neurosecretory material to the corpora cardiaca but through this organ to the aorta. In other words, the aortal wall has taken over the function of the corpora cardiaca as a neurohaemal organ (Dogra, 1967).

Other neurohaemal organs have been found along the medial nervous system in Carausius morosus (Phasmida), Schistocerca gregaria (Orthoptera), and Periplaneta americana and Leucophaea maderae (Dictyoptera) (de Bessé, 1966, 1967; Brady and Maddrell, 1967); along the peripheral abdominal nerves in Rhodnius prolixus (Hemiptera) (Maddrell, 1966); and along the nervi corporis allati II in Gryllodes sigillatus (Orthoptera) (Awasthi, 1968).

The intrinsic cells of the corpora cardiaca secrete a number of hormones. Among these is a hyperglycaemic factor (Steele, 1961) and a polypeptide that stimulates pericardial cells to release an amine that accelerates the heart beat (Davey, 1961).

A pair of nervi corporis allati (NCA) pass from the corpora cardiaca to the corpora allata (Fig. 2). In some species, these nerves contain neurosecretory

material and, thus, presumably arise in the brain; perhaps they are extensions of the NCC. In some insects there are a second pair of NCA that travel between the corpora allata and the suboesophagial ganglion (Awasthi, 1968).

The corpora allata secrete one or, possibly, two hormones; there are two main effects of this (these) hormones. In the larva, the juvenile hormone represses the appearance of adult characteristics at moulting; in the adult, a gonadotrophic hormone induces vitellogenesis (Wigglesworth, 1954, 1965) (see below, p. 28). As discussed below, p. 32, most of the present evidence supports the contention that these two hormones are identical, though this fact has not yet been proven.

It is, perhaps, somewhat out of place to discuss the thoracic glands (Fig. 2) with the retrocerebral system as these are not, except in the higher Diptera (see below, pp. 23-4-5) associated morphologically. However, they are functionally related and this discussion of the generalized insect endocrine system will end with a description of them.

These glands, usually found in the thorax, are quite loosely aggregated and closely associated with

tracheae. Upon stimulation by the brain hormone, they produce the hormone ecdysone which induces ectodermal cells to produce new cuticle. The result is a moult (Wigglesworth, 1954). In most insects, the thoracic glands are resorbed in the adult; however, in those apterygotes that continue to moult as adults (Thysanura), they persist (Wigglesworth, 1965).

B. The Retrocerebral System in the Diptera

The Order Diptera is composed of three suborders: the Nematocera, the Brachycera, and the Cyclorrhapha. These are generally thought of as being phylogenetically primitive, intermediate, and advanced, respectively (Imms, 1957).

1. Suborder Nematocera: *Tipula* sp. (Fig. 3)

Cazal (1948) distinguishes two types of Nematoceran retrocerebral systems: that of the Family Tipulidae (the crane flies) and that of other families. His description of that of *Tipula* sp., the type genus of the Family Tipulidae, is described here.

In the brain are located medial and lateral neurosecretory cells. Cazal (1948), it must be emphasized,

did not use the term "neurosecretory cell" in his study; nor did he use PAF, CAHP, or other neurosecretory stains (see above, p. 15). These cells he describes as being "cellules chromophiles" because of their red staining with ponceau in the trichrome technique of Masson. From his descriptions of their cytology and of their location, it is clear that they represent the medial and the lateral neurosecretory cells.

Paired NCC are formed from axons of these neurosecretory cells; they run between the brain and the paired corpora cardiaca. The corpora cardiaca are comma-shaped organs, rich in glial cells, nerve fibres, and large chromophil intrinsic cells. Their posterior prolongations (Fig. 3) contain processes of these intrinsic cells, as well as glial cells and nerve fibres. The corpora cardiaca are entirely separate from the aorta wall.

The paired corpora allata are made up of a large number of secretory cells, interspersed with chromophil secretory droplets.

2. Suborder Nematocera: Aedes aegypti (Fig. 4)

As an example of the remainder of the Nematocera, a description will be given of the retrocerebral system of the yellow-fever mosquito, Aedes aegypti, after that of Burgess and Rempel (1966).

Axons of cerebral neurosecretory cells form a pair of NCC that pass posteriorly to an organ called the "dorsal mass." This is fused to the hypocerebral ganglion and is closely applied to the aorta wall. It contains droplets of PAF-positive secretion and is thus thought to represent that part of the corpus cardiacum that stores and releases cerebral neurosecretion.

A pair of nerves pass posteriorly from each dorsal mass to the "gland complex" in the thorax. These nerves also contain neurosecretory material, presumably from the brain.

Each gland complex contains a region which is clearly the corpus allatum, some cells that may be intrinsic secretory cells of the corpus cardiacum, and, in the larva, cells that are thought to represent those of the thoracic glands.

3. Suborder Brachycera: Tabanus bovinus (Fig. 5)

The horse-fly, Tabanus bovinus, is representative of the Suborder Brachycera; its retrocerebral system was found to be identical to that of four other brachycerans by Cazal (1948), on whose paper the following description is based.

Cerebral neurosecretory cells give off axons that form a pair of NCC. These pass posteriorly, embedded in the aorta wall, and lead to the paired, elongate corpora cardiaca. The corpora cardiaca are each composed of about twelve large chromophil cells and are closely applied to, but separate from the aorta wall.

A pair of NCA lead from the corpora cardiaca to the unpaired corpus allatum which touches the dorsal wall of the aorta. This small organ is composed of many cells with numerous mitochondria.

4. Suborder Cyclorrhapha: Larval endocrine organs

The above accounts of the retrocerebral systems of the Nematocera and the Brachycera apply mainly to

the adult insects. The chief difference between larval and adult retrocerebral systems in insects is the presence, in the larva, of the thoracic glands or their equivalents. Thoracic glands have not been found in Tipula (Novák, 1966). In Aedes, as mentioned above on page 22, they form a part of the larval "gland complex." Novák (1966) mentions the presence in Tabanus, of a thoracic gland (peritrachial gland), but gives no details on its location or its structure.

The larval retrocerebral system in the Cyclorrhapha is quite specialized, consisting of a ring of tissue encircling the aorta. This is located directly above the brain in Calliphora vomitoria (Burtt, 1937), above and anterior to the brain in Drosophila melanogaster (King et al., 1966) and Lucilia sericata (Day, 1943a), and above and posterior to the brain in Eristalis tenax (Cazal, 1948). This ring, discovered by A. Weissmann (1864), is called "Weissmann's ring" or the "ring-gland."

The ring-gland is made up of four regions. The unpaired corpus allatum is located in the mid-dorsal region; the corpus cardiacum and the hypocerebral ganglion are fused and are located in the mid-ventral

region. The two lateral sides of the ring-gland each consists of a NCA between the corpus cardiacum-hypocerebral ganglion and the corpus allatum, and large cells thought to represent those of the thoracic glands (Wigglesworth, 1954; Novák, 1966) (Fig. 6).

In the pupal stage, the lateral portions of the ring-gland begin to degenerate (as do the thoracic glands of other insects); this degeneration is completed early in the adult stage (E. Thomsen, 1942) leaving the corpus allatum, the fused corpus cardiacum-hypocerebral ganglion, and the NCA between the two. This is the condition in the adult (E. Thomsen, 1942).

5. Suborder Cyclorrhapha: Calliphora erythrocephala (Fig. 7)

The retrocerebral system of the adult blowfly, Calliphora erythrocephala, has been the object of many morphological and physiological investigations. Some of the physiological investigations will be described in Section C of this chapter.

Neurosecretory cells in the pars intercerebralis give off axons that, after crossing over, leave the brain forming a pair of short NCC (E. Thomsen, 1954; M. Thomsen, 1965).

The two NCC join the recurrent nerve to form the unpaired cardiacum-recurrent nerve to the fused corpus cardiacum-hypocerebral ganglion (E. Thomsen, 1942). The corpus cardiacum portion of this complex contains the endings of neurosecretory axons from the brain, along with intrinsic cells that show ultrastructural evidence of being neurosecretory (Normann, 1965). Paired NCA arise from it, travel around the aorta, and innervate the corpus allatum.

The corpus allatum is an unpaired gland that shows a definite cycle of secretion correlated with the ovarian cycle (E. Thomsen, 1942) (see below, p. 28).

6. Suborder Cyclorrhapha: Glossina morsitans (Fig. 8)

The close similarity between the reproductive systems of the tsetse flies and the Pupipara (Wigglesworth, 1965, p. 660) is interesting from the point of view of convergent evolution. It might be asked whether this similarity extends to the endocrine systems of these two groups. The following description of the retrocerebral system of Glossina morsitans is based on a study by Langley (1965). As will be seen below (Chapter IV), this system is, in fact, morphologically quite

different from that of Melophagus; i.e., the convergence between these two dipterans ends with their reproductive systems.

In the brain of the tsetse fly are medial and lateral neurosecretory cells. The axons from these cells give rise to a pair of NCC that emerge from the postero-ventral edge of the brain and pass along the dorsal oesophageal wall to the corpora cardiaca. One NCC, usually the right one, fuses with the recurrent nerve behind the brain.

The corpora cardiaca, which are elongate organs fused posteriorly, are closely associated with the aorta wall. They contain irregularly shaped intrinsic cells with well-defined cell borders. Secretions from the cerebral neurosecretory cells are stored in the tissues of the cardiaca close to the wall of the aorta.

The corpus allatum is a spherical unpaired organ on the dorsal wall of the aorta over the paired anterior portion of the corpus cardiacum. Its cells are irregularly shaped with indistinct cell boundaries.

C. The Insect Retrocerebral System and Reproduction

In the thirty-two years since Wigglesworth (1936) first demonstrated an endocrine control of insect reproduction, this topic has been the subject of numerous investigations. The volume of the papers published on this topic necessitates that its review in this thesis be quite brief and superficial. Review articles have been written recently by Highnam (1963), Davey (1965), and Engelmann (1968), to which the reader is referred for more detailed accounts on the subject.

In 1936, Wigglesworth showed that, by decapitating fed adult female Rhodnius prolixus (Hemiptera) posterior to the corpus allatum, yolk deposition is prevented and immature oocytes are resorbed; decapitation anterior to the corpus allatum does not have this effect. He concluded that a hormone controlling vitellogenesis is secreted from the adult corpus allatum.

Ellen Thomsen, in a series of experiments on the blowfly Calliphora erythrocephala, showed that the corpus allatum has an effect on vitellogenesis (1942) but that that of the medial neurosecretory cells is greater (1952).

One or both of these two endocrine systems have

been implicated in the control of vitellogenesis in most insects investigated. However, it soon became apparent that non-endocrine factors, such as nutrition and mating, are of equal or greater importance in the control of ovary development.

One example which has been quite well worked out is that of the blowfly, Calliphora erythrocephala. As mentioned above (p. 28), the corpus allatum and the medial neurosecretory cells of these flies have been implicated in the control of vitellogenesis. A source of protein in the diet is also required (Thomsen and Møller, 1963).

The secretion of the corpus allatum depends on the presence in the haemolymph of the fly of "protein metabolites" (presumably free amino acids). Prior to vitellogenesis, the flies eat meat and the haemolymph protein concentration increases; the corpus allatum then increases in size and activity. During vitellogenesis, when proteins are removed from the haemolymph and are used in yolk formation, the corpus allatum decreases in volume and activity. If excessive protein is fed the fly or if the fly is ovariectomized, this decrease in allatum volume does not take place because

the quantity of "protein metabolites" in the haemolymph remains high (Strangways-Dixon, 1961, 1962).

Thomsen and Møller (1963) state that the synthesis of intestinal proteases in the blowfly is dependent on a hormone from the medial neurosecretory cells. They feel that, since proteases are themselves proteins, the mode of action of the medial neurosecretory cells may be to stimulate protein synthesis in general.

Davey (1965) proposed an interpretation of the results of these two groups of experiments.

The eating of meat (protein) by the blowfly in some way stimulates the medial neurosecretory cells to produce their hormone. This hormone stimulates gut protease synthesis and, thus, the digestion of the protein meal.

Digestion of protein results in the presence of "protein metabolites" in the haemolymph; these stimulate the corpus allatum to secrete its hormone which induces the ovaries to take up protein for manufacturing yolk. In addition, as the primary effect of the medial neurosecretory cells' hormone is to stimulate protein synthesis, it aids in the synthesis of these yolk

proteins. Vitellogenesis removes the "protein metabolites" from the haemolymph; this "shuts off" the corpus allatum. The net result of this is ovarian development.

Highnam and his associates have given evidence that a similar system is in operation in the desert locust, Schistocerca gregaria. In this case, however, the primary stimulus for the release of cerebral neurosecretory material is thought to be mating (Highnam, 1962). Here, as in Calliphora, the medial neurosecretory cells regulate protein synthesis and the corpora allata regulate protein uptake by the developing oöcytes (Highnam et al., 1963).

Of interest as regards the endocrine control of reproduction in the viviparous sheep ked, is that of the viviparous cockroaches, Leucophaea maderae and Diploptera punctata. In these insects, the corpora allata, but not the medial neurosecretory cells, are necessary for egg maturation (Engelmann, 1968).

In virgin females of these two species, the corpora allata are under inhibition by nervous impulses from the brain and suboesophageal ganglion via the NCA. These inhibitory impulses are abolished by copulation; the corpora allata then secrete their hormone and the

ovaries develop (Engelmann, 1960).

In these species, the oötheca is retained for a considerable length of time in the genital ducts. Thus, it is important that further egg development be halted until parturition occurs. This retardation of further ovarian growth is brought about by the resumption of the inhibitory nervous impulses to the corpora allata from the brain. This reimposition of inhibition may be brought about by one of two means: nervous (via the ventral nerve cord) or humoral (via a secretion from the ootheca) (Engelmann, 1962).

Parturition has the same effect that mating has on virgin females, i.e., it abolishes the inhibition of the corpora allata and allows for the maturation of the next batch of eggs.

The endocrine control of reproduction in the viviparous cockroaches will be of some interest later (p. 74) when the control of reproduction in the ked is discussed.

Before ending this brief review of the endocrine control of insect reproduction, I feel it would be well to say a few words on the identity of the gonadotrophic

hormones. To my knowledge, there is no evidence of the identity of the medial neurosecretory cells' gonadotrophic hormone. However, the corpus allatum hormone has elicited much interest by its apparent similarity to the juvenile hormone (see above, p. 19). As discussed by Davey (1965), although much evidence has accumulated recently linking the two hormones, it has not yet been proven that they are identical. With the identity of the juvenile hormone now established (Williams, 1967), this problem may be close to a final solution.

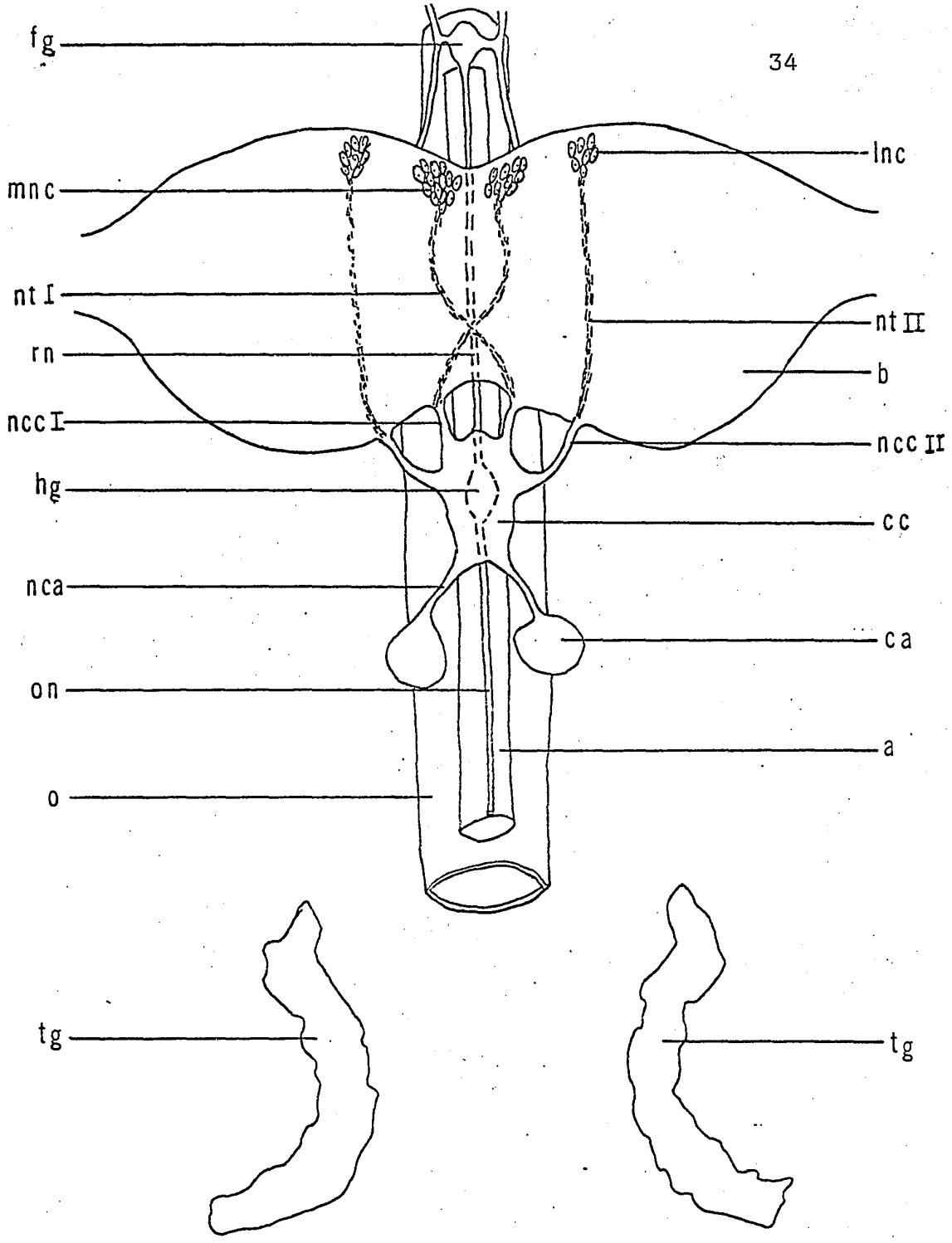


Figure 2. The retrocerebral system of a "generalized" insect. (After Highnam, 1967).

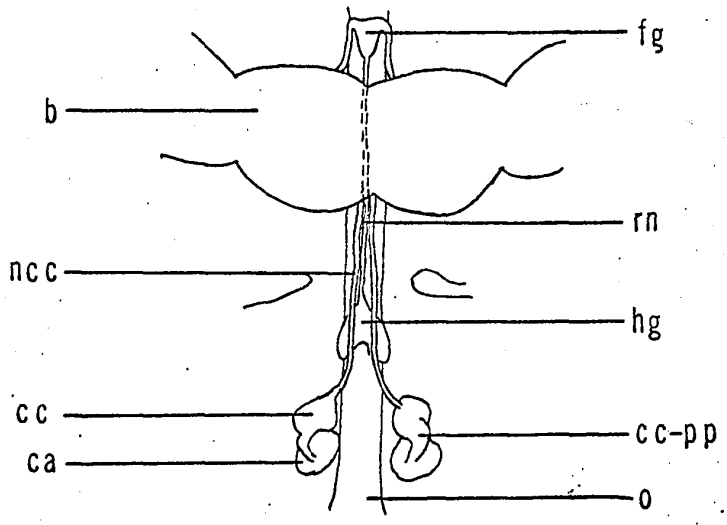


Figure 3. The retrocerebral system of the adult of Tipula sp. (Diptera : Nematocera). (After Cazal, 1948).

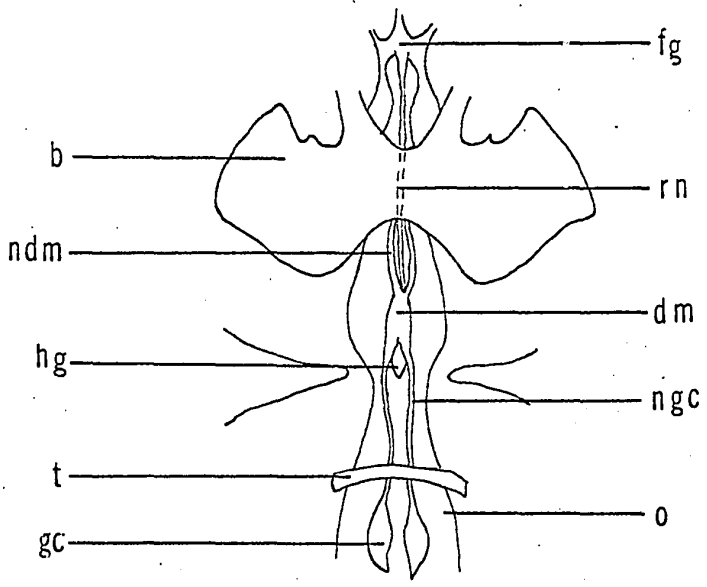


Figure 4. The retrocerebral system of the adult of Aedes aegypti (Diptera ; Nematocera). (After Burgess and Rempel, 1966).

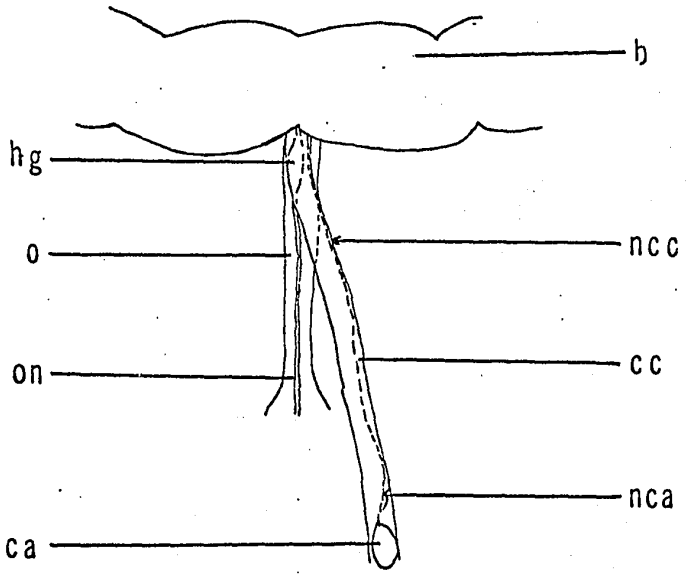


Figure 5. The retrocerebral system of the adult of Tabanus bovinus (Diptera : Brachycera). (After Cazal, 1948).

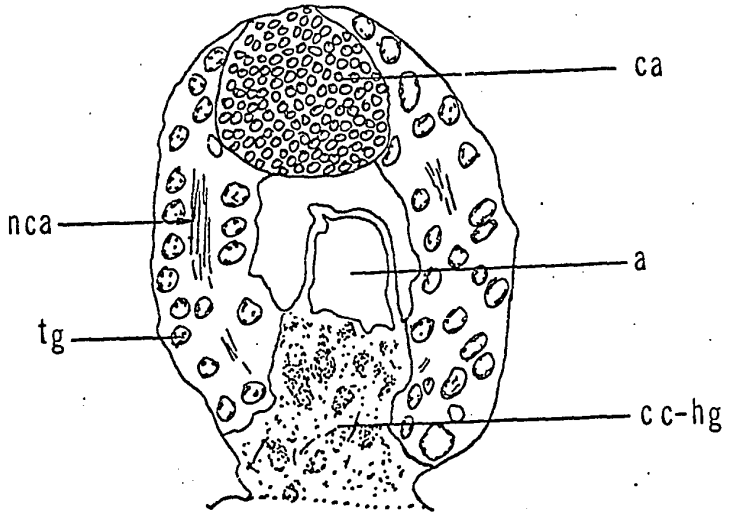


Figure 6. The ring-gland of a young pupa of Eristalis tenax (Diptera : Cyclorrhapha). (After Cazal, 1948).

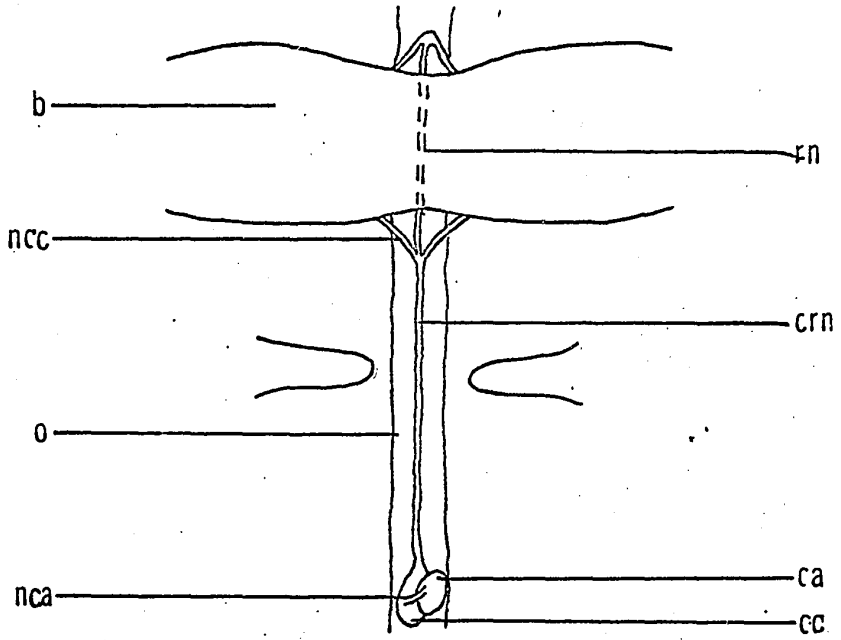


Figure 7. The retrocerebral system of the adult of Calliphora erythrocephala (Diptera : Cyclorrhapha). (After the description given in E. Thomsen, 1942).

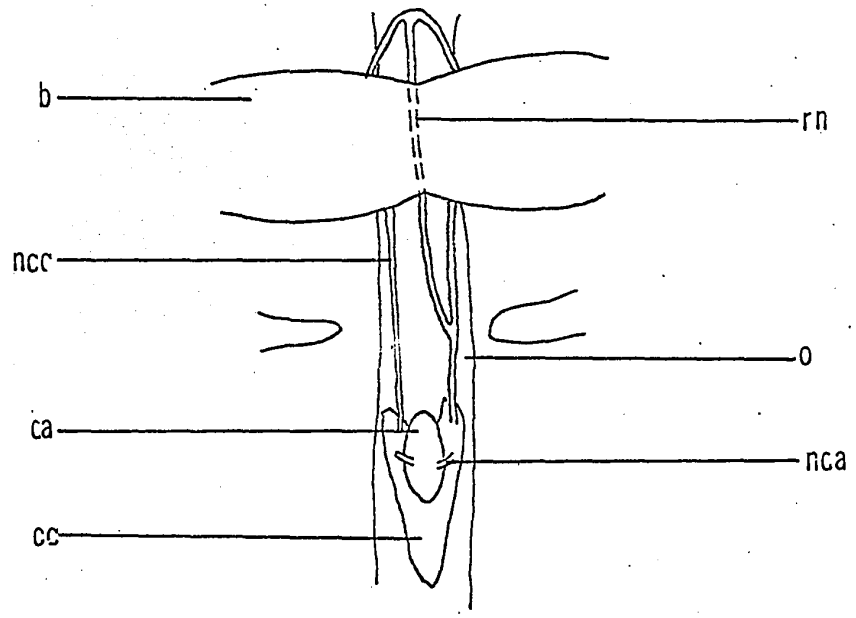


Figure 8. The retrocerebral system of the adult of Glossina morsitans (Diptera : Cyclorrhapha). (After the description given in Langley, 1965).

CHAPTER IV

THE KED RETROCEREBRAL SYSTEM

A necessary preliminary to any study of the endocrine control or reproduction in an insect is, of course, a knowledge of the morphology of that insect's retrocerebral system. For this reason, a study was made of this system in Melophagus. Conclusions are based on examination of 148 keds of both sexes.

In the supraoesophageal ganglion (the "brain" sensu strictu), between the two hemispheres, is a region characterized by the presence of large nerve cells. This region (Figs. 10 and 18) comprises the pars intercerebralis. However, none of these cells is recognizable as being neurosecretory by the light microscopical techniques used (PAF, azan, CAHP).

After staining with the PAF technique, the large nerve cells of the pars intercerebralis stain orange or, if permanganate oxidation is overly prolonged, light purple. This purple colour is not at all like the dark purple seen in the medial neurosecretory cells of

Rhodnius prolixus brains stained simultaneously with PAF.

Sections of sixty-six keds of both sexes and all developmental stages were stained with PAF and examined closely to determine whether small amounts of neurosecretion are present in the nerve cells of the pars intercerebralis. If secretion is continuous, stainable neurosecretory granules would not be expected to be present in large amounts at any one time; however, small amounts might be present. No evidence is found of any PAF-positive neurosecretory granules.

This is further substantiated by the fact that the few larvae and pupae that were processed and stained with PAF also did not appear to possess neurosecretory cells.

The results of azan staining are less conclusive. Some of the pars intercerebralis nerve cells occasionally stained red; however, the appearance of these cells is not similar to that of azan positive neurosecretory cells described by de Bessé (1967, Figs. 1-4) in Leucophaea maderae and Periplaneta americana. The red colour is probably due to incomplete differentiation of the azocarmine during staining.

CAHP staining was not at all successful, for unknown reasons.

Between the brain and the endocrine glands in the thorax are the paired nervi corporis cardiaci (NCC) (Figs. 9, 11-16). These nerves presumably are formed from axons of the pars intercerebralis nerve cells (see below, p. 72). They emerge from the brain alongside the oesophagus as it emerges from the oesophageal channel and, passing alongside the oesophagus, travel posteriorly and attach to the corpus cardiacum (Figs. 20 and 21).

The corpus cardiacum (Figs. 9, 15-17) is an unpaired gland composed of large intrinsic cells, smaller cells, and nerve fibres. There is no evidence of any stored neurosecretory material, an observation that supports my contention that there are no cerebral neurosecretory cells. This organ is entirely separate from the aorta wall (Figs. 15-17).

The intrinsic cells (Figs. 20-23) have vacuolar cytoplasm, implying that they are actively synthesizing and storing a hormone which is soluble in the fluids used in processing the tissues. Nuclei are generally smaller than those of the allatal cells and cell

boundaries are distinct, especially in Zenker's fixed material stained with azan.

The remainder of the corpus cardiacum is confusing, to say the least. Nerve fibres are present, but the small cells that are associated with them are puzzling (Figs. 21-23). I feel that they may be glial cells or small neurons. This will be discussed in more detail below (p. 73).

Between the corpus cardiacum and the paired corpora allata are the nervi corporis allati. These nerves are often very reduced and may be unrecognizable. On the other hand, in a few keds with corpora allata located an unusually great distance from the corpus cardiacum, they may be quite prominent.

The corpora allata (Figs. 9, 14-16, 24) are located above the corpus cardiacum and lateral to and separate from the aorta. They consist of a variable number of secretory cells (Fig. 24) which, in most keds studied, male and female, appear to be actively synthesizing, storing, and releasing hormones. This is discussed in more detail in the next chapter and in Chapter VI.

There are two other features of the ked retrocerebral system that deserve mention. One is the large suboesophageal ganglion; the other is the lack of a recurrent nerve.

In the ked, there is but little external differentiation between the brain and the suboesophageal ganglion. It is evident, however, in saggital sections passing through the oesophagus and the oesophageal channel (Fig. 19). Here it is often observed that the suboesophageal ganglion is, in fact, larger than the brain.

Careful examination of the ked oesophageal channel has failed to reveal any trace of a recurrent nerve; there is also no trace of it between the brain and the corpus cardiacum. In most other insects, this nerve runs through and behind the brain dorsal to the oesophagus. In the Cyclorrhapha, it often fuses with one or both of the nervi corporis cardiaci posterior to the brain (see above, pp. 26 and 27). It is formed anteriorly from the frontal ganglion and forms the hypocerebral ganglion at about the level of the corpus caridacum (Fig. 2).

In the ked, no frontal ganglion is seen by dissections or in histological preparations.

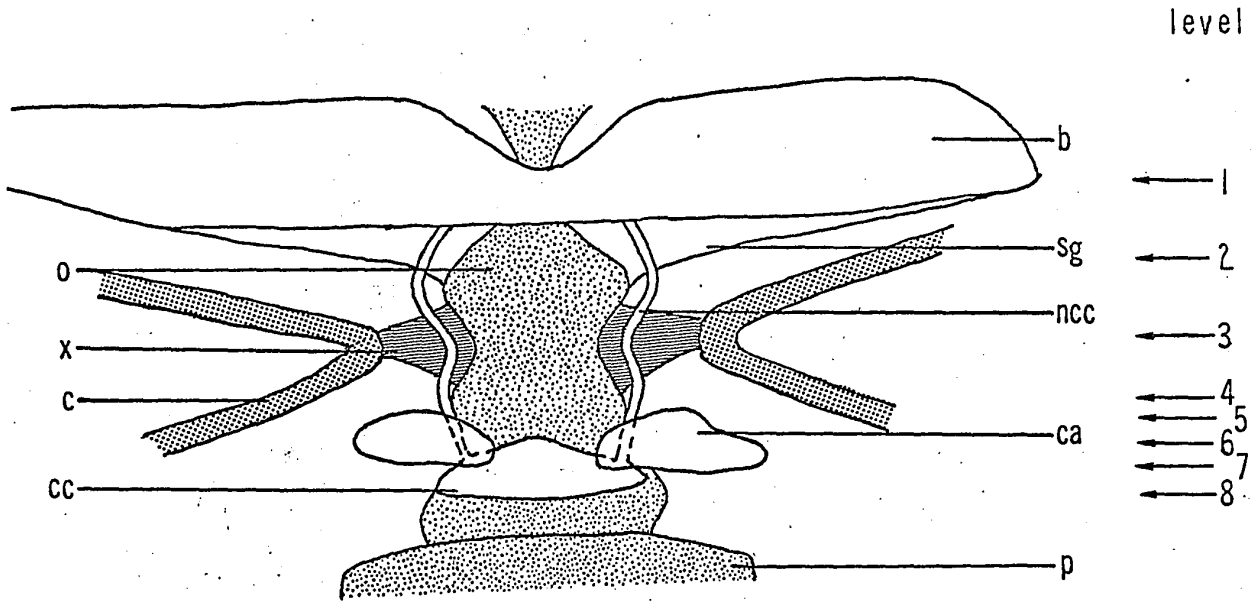


Figure 9. The retrocerebral system of the adult sheep ked, Melophagus ovinus, dorsal view. Levels "1" through "8" refer to Figures 10 through 17.

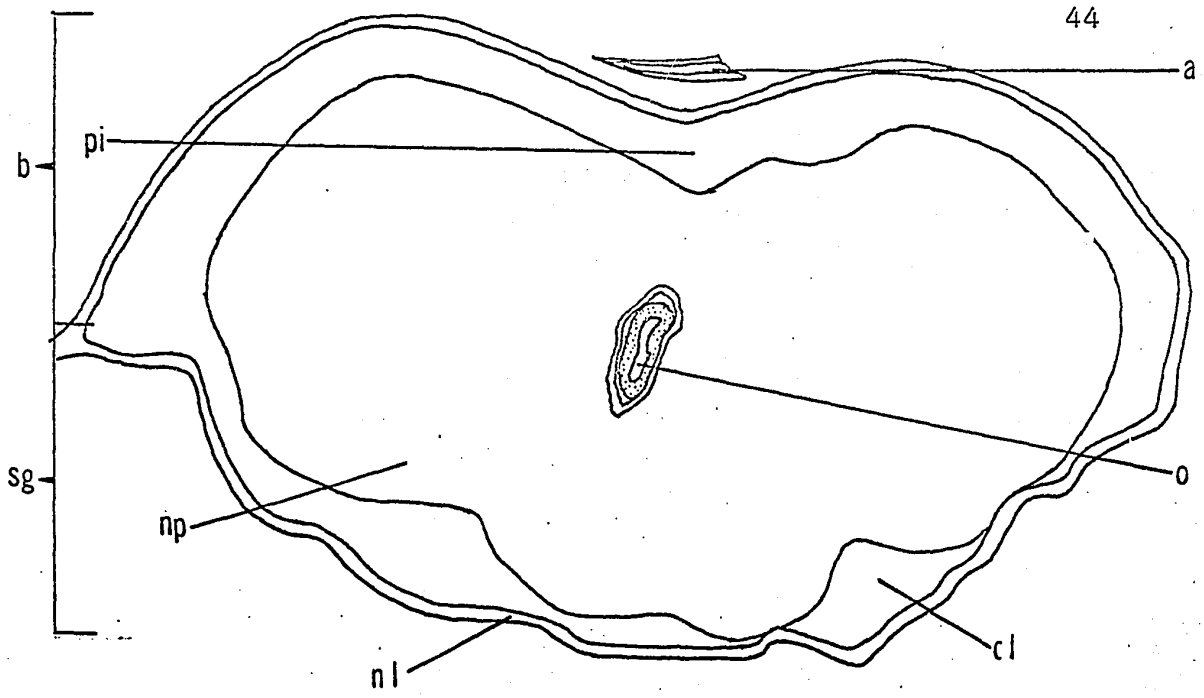


Figure 10. Level 1. Cross section through a ked at the level of the brain and the suboesophageal ganglion.

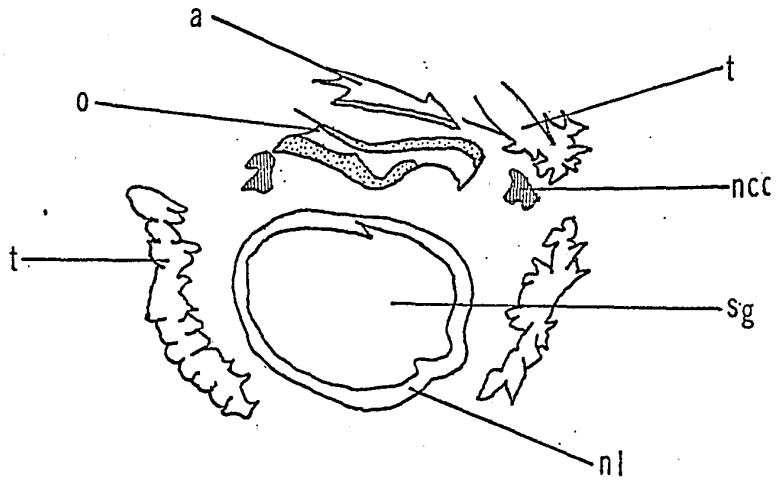


Figure 11. Level 2. Cross section through a ked posterior to the brain but anterior to the level at which the suboesophageal ganglion merges with the ventral nerve cord. Note the newly formed nervi corporis cardiaci (ncc).

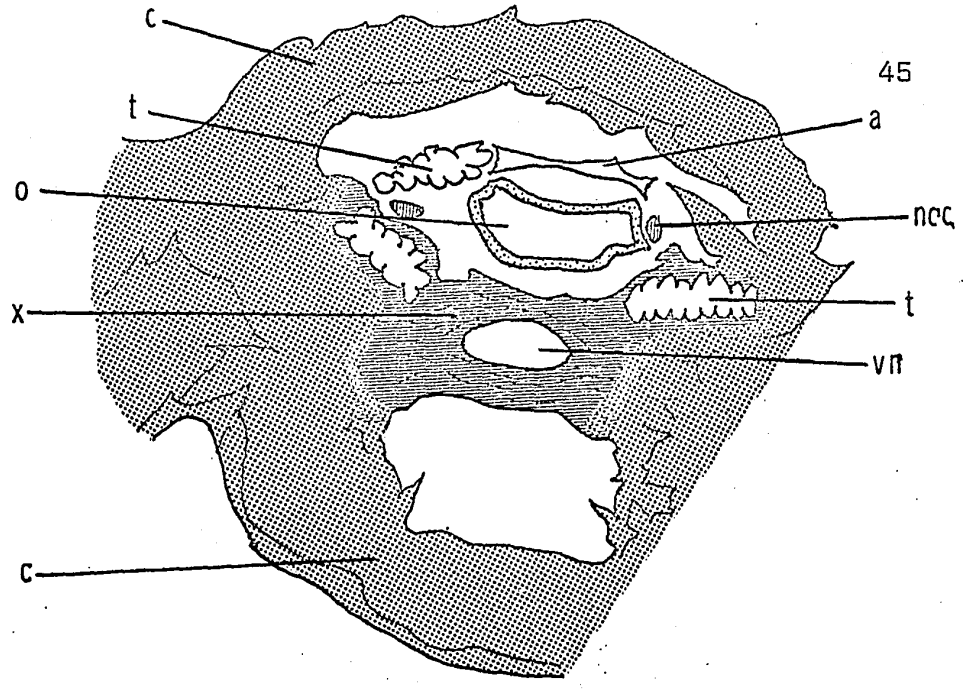


Figure 12. Level 3. Cross section through a ked in the area of the structure around the ventral nerve cord in the neck (Appendix E). Note the close association of this structure (x) with cuticle (c) and with tracheae (t).

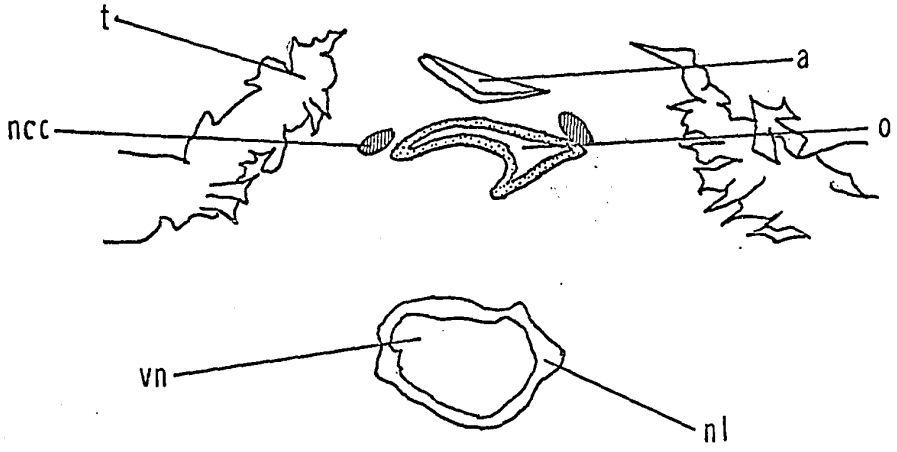


Figure 13. Level 4. Cross section through a ked between the neck and the corpora allata and corpus cardiacum.

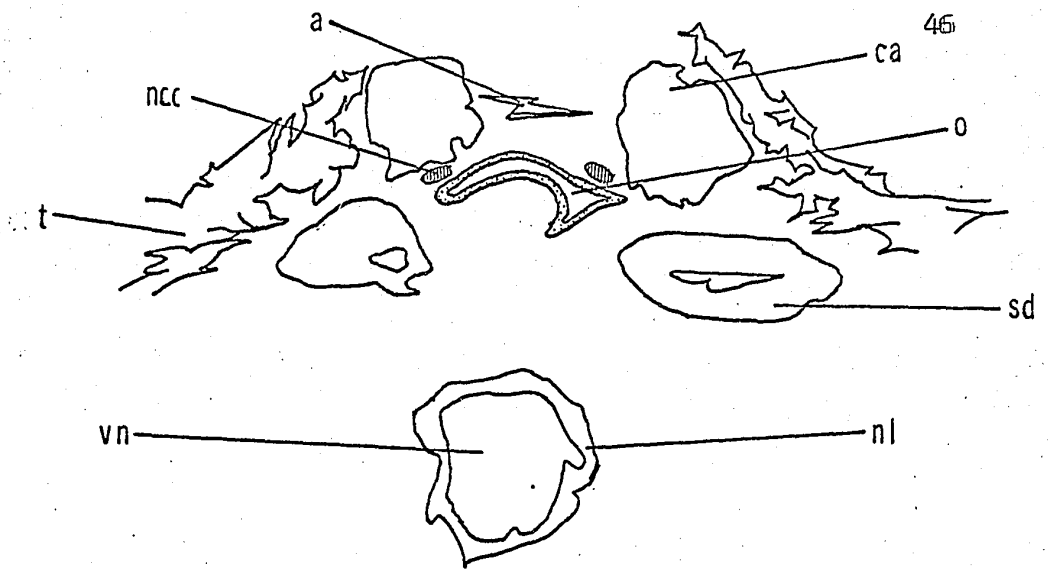


Figure 14. Level 5. Cross section through a ked at the level of the anterior end of the corpora allata.

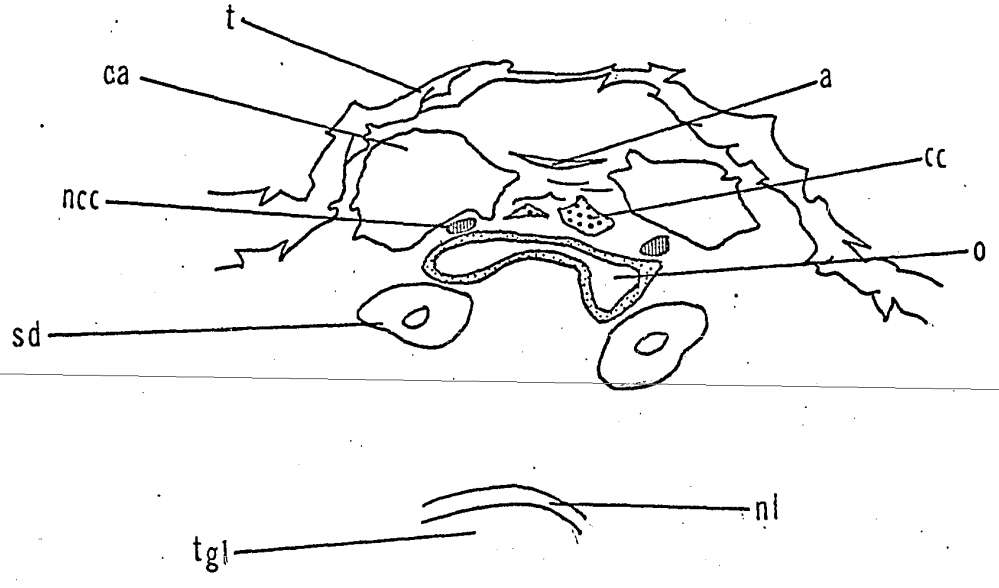


Figure 15. Level 6. Cross section through a ked midway through the allata and at the anterior end of the cardiacum.

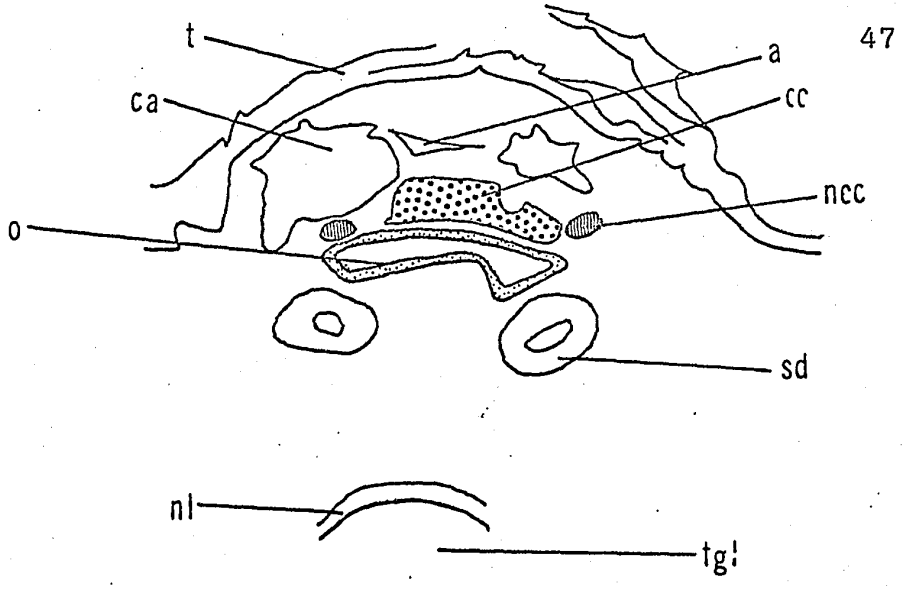


Figure 16. Level 7. Cross section through a ked at the posterior end of the allata and midway through the cardiacum.

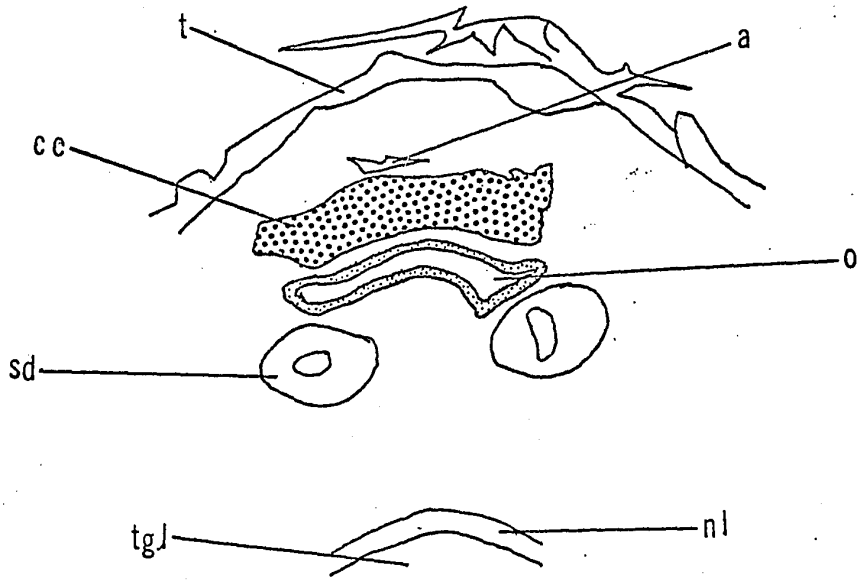


Figure 17. Level 8. Cross section through a ked posterior to the allata. Note that the nervi corporis cardiaci have merged with the corpus cardiacum.

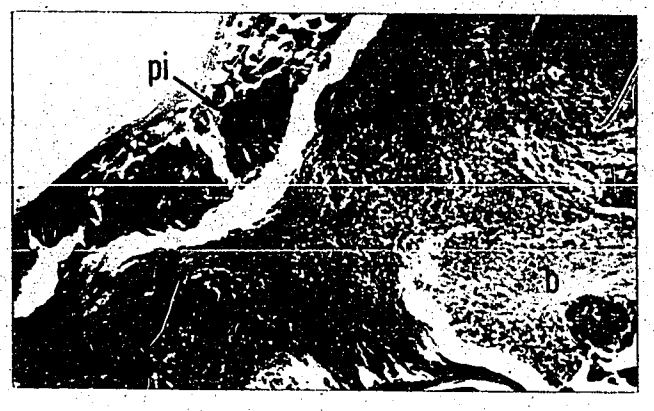


Figure 18. Pars intercerebralis of a ked stained with PAF. Note the large nerve cells. Fix: Bouin's. x420.

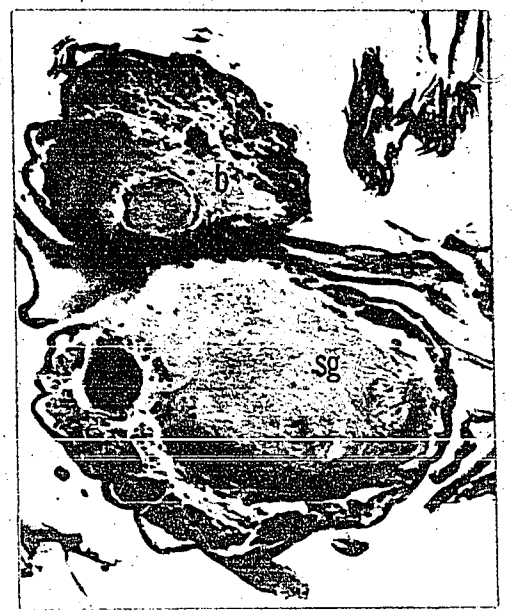


Figure 19. Nearly saggital section through the brain and suboesophageal ganglion of a ked. Note the large size of the suboesophageal ganglion. Bouin's, PAF. x220.

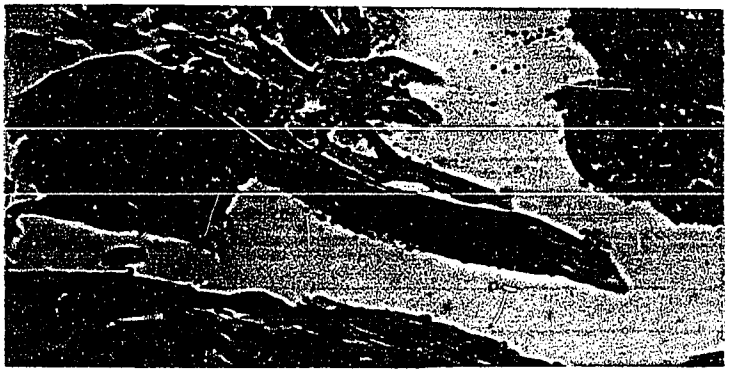


Figure 20. Saggital section of a ked showing NCC entering corpus cardiacum. Note nerve fibres and intrinsic secretory cells. Zenker's, PAF without oxidation. x640.

Figure 21. Oblique frontal section of a male ked's thorax showing one of the NCC entering the corpus cardiacum. Bouin's, Hansen's. x400.

Figure 22. Saggital section of the ked corpus cardiacum showing very clearly the nerve fibres and intrinsic secretory cells and, less clearly, the small cells. Bouin's, PAF. x 330.

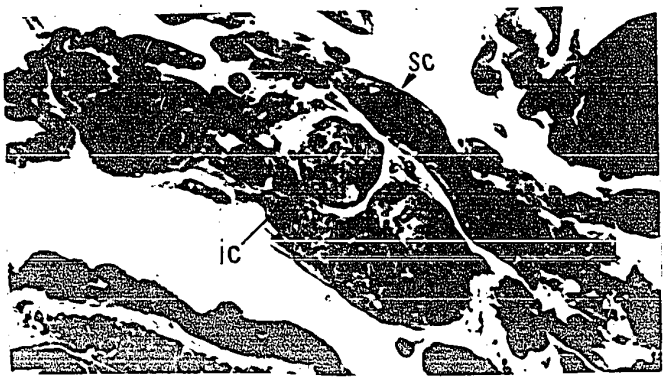


Figure 23. Details of corpus cardiacum. Note the "small cells" and several intrinsic secretory cells. Zenker's Heidenhain's azan. x770.

Figure 24. Section through the corpus allatum of a female ked. Bouin's, Heidenhain's azan. x820.

CHAPTER V

THE KED RETROCEREBRAL SYSTEM AND REPRODUCTION

The relationship of the activity of the ked's retrocerebral system to its reproductive cycle was studied after the morphology of the retrocerebral system had been worked out. The terms "reproductive cycle" and "ovarian cycle" are defined in the ked as the time involved in maturing each oocyte. In other words, they represent the time between the start of egg maturation and ovulation. They are described in more detail in the next section of this chapter.

A. The Reproductive Cycle

Studies on the endocrine control of insect reproduction usually begin with observations of the normal cycles of the suspect organs and then graduate to extirpation and implantation experiments. In this investigation, only the former technique was found to be feasible.

Extirpation and implantation experiments require

that the experimental animals be kept confined for observation. Keds, because of their ectoparasitic existence on sheep, are very difficult animals to observe in this way. Nelson (1955) succeeded in feeding them through a membrane and Evans (1946, 1950) successfully confined them on sheep in a knitted cage. However, both of these methods would be quite difficult to use to raise keds for experimentation in numbers sufficient for statistical analysis.

With this in mind, it was decided to correlate the reproductive cycle in the normal ked with the secretory cycle of its endocrine system. To indicate the stage of the reproductive cycle, the length of the larva in the female's uterus was measured. Pratt (1899) made the following observation: ". . . when the larva [in the uterus] has attained its maximum size and is ready to be born, the largest ovum . . . has . . . attained full size and is ready to be extruded. . . . The larva in the uterus is then born and that organ being emptied, soon afterward the ripe ovum passes in its turn from the ovary through the receptaculum seminalis, where it is fertilized, into the uterus." According to Ulrich (1963), vitellogenesis and

development of another oöcyte then begin. Thus, the ovarian cycle and the gestation period very closely parallel one another, so the growth of the larva in the uterus may be taken as an indication of the growth of the oocyte in the ovary.

The problem now becomes one of defining more precisely the relationship between the length of the larva and the stage of the reproductive cycle. This was determined indirectly as follows:

Each female ked was fixed in modified Bouin's fluid as in the morphological study (Appendix B) and her larva was later dissected out of the uterus in 70% alcohol. The length of the larva was measured by projecting its image with a projecting microscope, measuring the image, and calibrating the image with a stage micrometer. The ked was then assigned to a group on the basis of the length of her larva, thus:

A < 1.00 mm	E 1.76-2.00 mm	H 2.51-2.75 mm
B 1.01-1.25 mm	F 2.01-2.25 mm	I 2.76-3.00 mm
C 1.26-1.50 mm	G 2.26-2.50 mm	K 3.01-3.25 mm
D 1.51-1.75 mm		L > 3.26 mm

As sampling of the keds was done as randomly as possible, it was reasoned that the proportion of the total population in each group should be equal to the

proportion of the total gestation period (and thus of the total ovarian cycle) spent in that group. For instance, 26% of the population belong to group B; therefore, 26% of the ovarian cycle is represented by group B. The length of time represented by a given group is designated by the percentage of the reproductive cycle that has passed at the beginning and at the end of that "age group."

Figures obtained are in Table 1.

B. Endocrine Activity During the Reproductive Cycle

Keds used for the determination of the reproductive cycle were also used in the study of the activity of the endocrine system. These had all been uniformly fixed in modified Bouin's fluid and were now processed as in the morphological study (Appendix B). They were sectioned at 8 μ and stained with paraldehyde fuchsin, azan, or Hansen's iron trioxyhaematin (Appendix B).

With the pars intercerebralis dismissed as a source of hormones detectable by the techniques used (Chapter IV), attention was focused on the activity of the corpora allata. This was investigated from two approaches: one qualitative and the other quantitative.

1. Qualitative approach to allatal activity

The qualitative approach consisted of studying the cytology of the allatal cells and attempting to correlate cytological evidence of activity, or lack of it, with the reproductive cycle. Such things as nuclear and cytoplasmic appearance and the presence or absence of vacuoles and nucleoli were observed.

The cytology of the allatal cells was found to be very variable, even between animals of the same group. To illustrate this, I will give examples from my notes:

Group A

Ked no.	
E45	Cell boundaries quite distinct, cytoplasm fairly vacuolar, nuclei large and regular with large nucleoli.
E59	Quite indistinct cell boundaries, numerous small vacuoles, large nuclei and nucleoli.
E82	Cell boundaries indistinct in places, very vacuolar cytoplasm, regular nuclei with large nucleoli.

Group B

E15	Distinct cell boundaries, cytoplasm vacuolar, nuclei varying in size and regularity with large nucleoli.
E25	Vaculoles small or lacking, large nuclei and nucleoli.

E26 Distinct cell boundaries, large peripheral vacuoles in most cells, smaller vacuoles in some, regular nuclei with large nucleoli.

Groups A and B represent fairly long durations (9% and 26% of the total reproductive cycle respectively). It might be asked whether an age group of shorter duration is more regular in the appearance of its allata.

That this is not so is shown, for instance, by a selection of observations from group H (6% of the total reproductive cycle):

Group H

E21 Many medium-sized vacuoles, medium-sized nuclei, large nucleoli.

E23 Large and small vacuoles that are not too numerous, large nuclei and nucleoli.

E44 Numerous small vacuoles, a few larger ones, nuclei medium-sized with large nucleoli.

Emerging from this is the observation that at all times during the reproductive cycle of the ked, the allatal cells give the appearance of being active, though the degree of activity is very variable. Nuclei are usually regular, often with quite large nucleoli, and never appear pycnotic. The cytoplasm is usually vacuolar, indicating that a secretion is there which is soluble in the liquids used to process the tissues.

Whether this secretion is being stored or released is not clear. One might postulate that secretions are continuously synthesized and stored until they fill the cytoplasm and are then released. In this case, large vacuoles would indicate late storage of secretions and small vacuoles would indicate release or early storage.

The question now arises as to whether these secretions are, in fact, responsible for the control of the female ked's reproductive cycle. This is, of course, impossible to say; however, approximately the same cytological picture as is found in the female ked is also found in the male. So, it seems that the corpora allata of both the male and the female sheep ked are continuously active.

2. Quantitative approach to allatal activity

The second approach to determining the activity of the corpora allata consisted of measuring and calculating four factors. These were: the volume of the allata, the estimated volume of the allatal cells, the cytoplasmic volume of the allatal cells, and the ratio of cytoplasmic to nuclear volume of the allatal cells. Methods used to determine these factors are described in Appendix C.

Novák (1966), Watson (1967), and Engelmann (1968) state, in effect, that the magnitude of these factors is directly related to the activity of the allata. In the case of the ked, where, as was just shown, the allata appear to be continuously active, it might be more accurate to say that high values for these factors imply storage of secretions, low values imply release.

a. Allatum volume

Many researchers have used the volume of the corpus allatum as a measure of the activity of the gland. It is by far the easiest parameter to measure; however, one must keep in mind the fact that it might be misleading as a measure of activity. A large gland containing many small cells might be less active than a smaller one with a few large cells. For this reason, I have not relied wholly on this one criterion.

The results of my measurements of allatal volume are summarized in Table 2, column 3, and in Figure 25. The most obvious feature is the great variation. This is not unexpected from the results of the cytological observations (above).

As seen in Figure 25, there does not seem to be

any pattern to the variations in allatal volume during the reproductive cycle. However, an analysis of variance (Appendix D) (Table 3) gives a result indicating that there is a significant difference, at the 0.05 level, between age groups ($0.05 > P > 0.02$). U-tests (Appendix D) quickly show that group F is the cause of this difference. There seems to be no logical reason for the low values obtained for group F and, if it is ignored, the analysis of variance shows that the variation within each age group is so great that no meaningful comparisons can be made (see below, ($P > 0.05$) (Table 3).

b. Allatum cell volume and cytoplasmic volumes

As indicated above, the other three criteria may be better indications of the activity of the corpora allata than their volume. The results of the measurements of the estimated volumes of the allatal cells and their cytoplasmic volumes are summarized in Table 2, columns 4 and 5, and in Figures 26 and 27. Again there seems to be no pattern to the large variation and the analysis of variance confirms it (Table 3). Once again, the variance within each age group is so great that comparisons between groups are meaningless ($P > 0.05$).

c. Ratio of cytoplasmic to nuclear volume

This last measurement might be the most meaningful of the four (Novák, 1966); however, once again, internal variation is too great for meaningful comparisons to be made ($P > 0.05$) Tables 2 and 3, Fig. 28).

The results of these qualitative and quantitative observations are discussed in more detail below (pp. 73-77).

TABLE 1. *Melophagus ovinus* reproductive cycle

(1)	(2)	(3)	(4)	(5)	(6)
A	8	9	1- 9	0.44	4
B	23	26	10-35	1.15	25
C	23	26	36-61	1.37	47
D	10	11	62-72	1.62	66
E	5	6	73-78	1.90	76
F	4	5	79-83	2.13	81
G	5	6	84-89	2.44	88
H	5	6	90-95	2.63	92
I	2	2	96-97	2.92	96
K	3	2	98-99	3.16	98
L	1	1	100	3.57	100
Totals	89	100			

(1) Group
(2) Number of insects in that group
(3) Percentage of observations in that group, rounded off to the nearest whole per cent. That of group K is altered by 1%.
(4) Percentage of the reproductive cycle that has passed at the beginning and at the end of that age group.
(5) Average length of larvae in that group (mm.)
(6) Average percentage of the reproductive cycle represented by that age group. This is a meaningless statistic in itself, and was calculated for ease in plotting data (Figs. 25-28).

TABLE 2. Means and standard deviations of the quantitative measurements of allatal activity

(1)	(2)	(3)	(4)	(5)	(6)
A	7	225±124	1.89±0.58	1.78±0.56	16.9 ±5.0
B	11	277± 67	2.49±0.62	2.35±0.59	19.3±10.0
C	10	320± 90	2.64±0.73	2.52±0.72	20.7 ±6.8
D	9	304± 61	2.39±0.74	2.12±0.68	16.0 ±5.1
E	5	310± 85	2.52±0.21	2.38±0.17	17.1 ±3.8
F	4	162± 35	1.75±0.39	1.65±0.37	15.6 ±2.6
G	5	281± 73	2.22±0.49	2.08±0.48	17.2 ±1.8
H	5	243± 75	1.94±0.28	1.84±0.28	18.1 ±3.6
I	2	227± 29	2.04±0.10	1.94±0.28	19.4 ±1.1
K	3	*311± 4	2.91±0.48	2.45±0.33	21.0 ±2.4

(1) Group
(2) Number of observations in that group (see (3))
(3) Volume of corpora allata (x 10^3 μ^3) (*Group K was figured from two observations)
(4) Estimated cellular volume of corpora allata (x 10^3 μ^3)
(5) Cytoplasmic volume of corpora allata (x $10^3\mu^3$)
(6) Ratio of cytoplasmic to nuclear volume

TABLE 3. Results of analysis of variance of quantitative measurements of allatal activity

	(1)	(2)	(3)	(4)	(5)
corpora allata volume	2.32	9	50	0.05-0.01	yes
cell volume	1.81	9	51	>0.05	no
cytoplasmic volume	0.92	9	51	>0.05	no
cytoplasmic: nuclear volume ratio	0.27	9	51	>0.05	no
corpora allata volume less group F	1.42	8	47	>0.05	no

(1) Value of F
(2) d.f. between groups
(3) d.f. within groups
(4) Probability
(5) Significant?

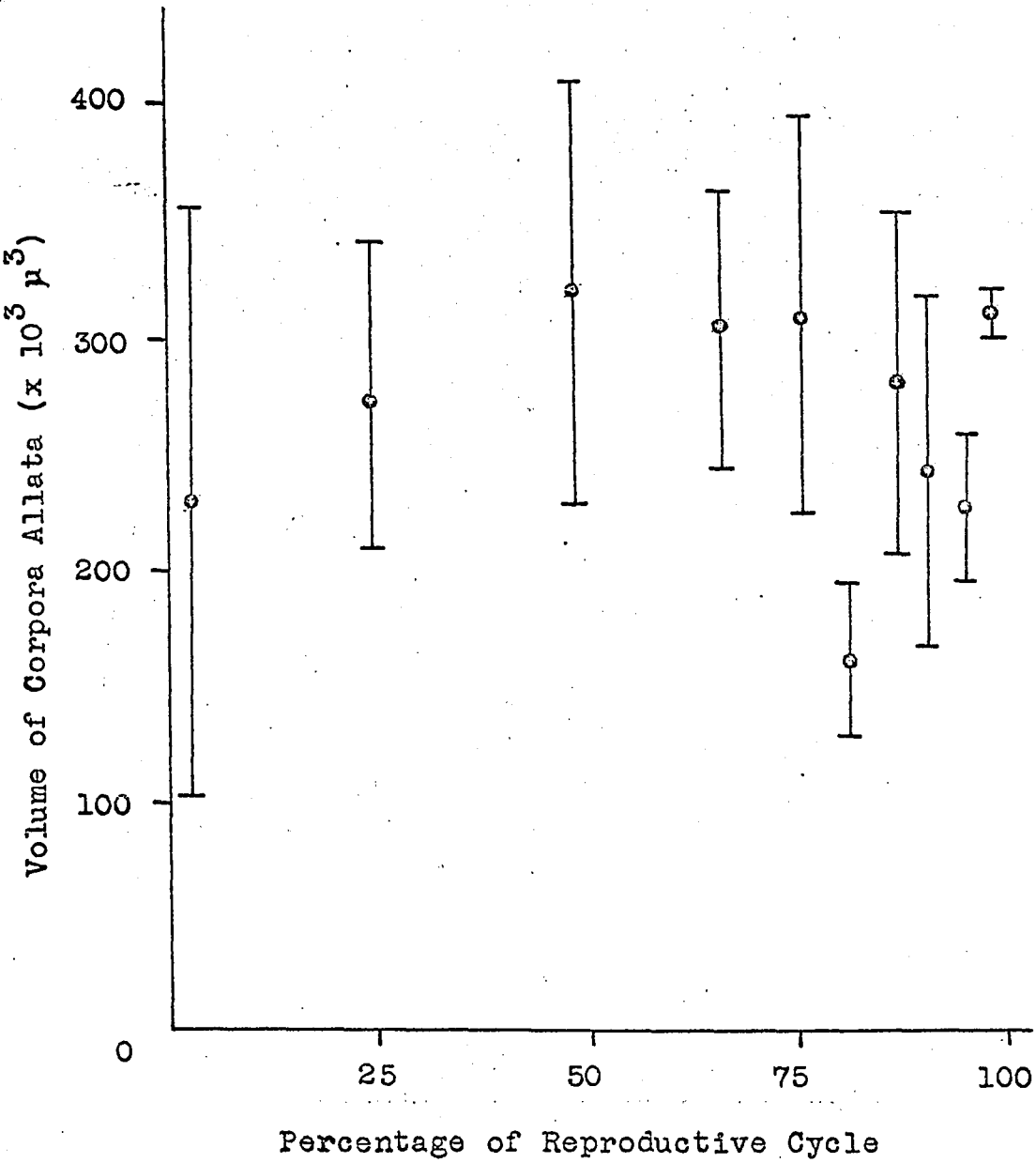


Figure 25. Graph showing the variations in the volumes of the corpora allata with the reproductive cycle of the sheep ked. The verticle lines in this and in the following three figures (26-28) represent the standard deviations of the observations. Note the large amount of variation.

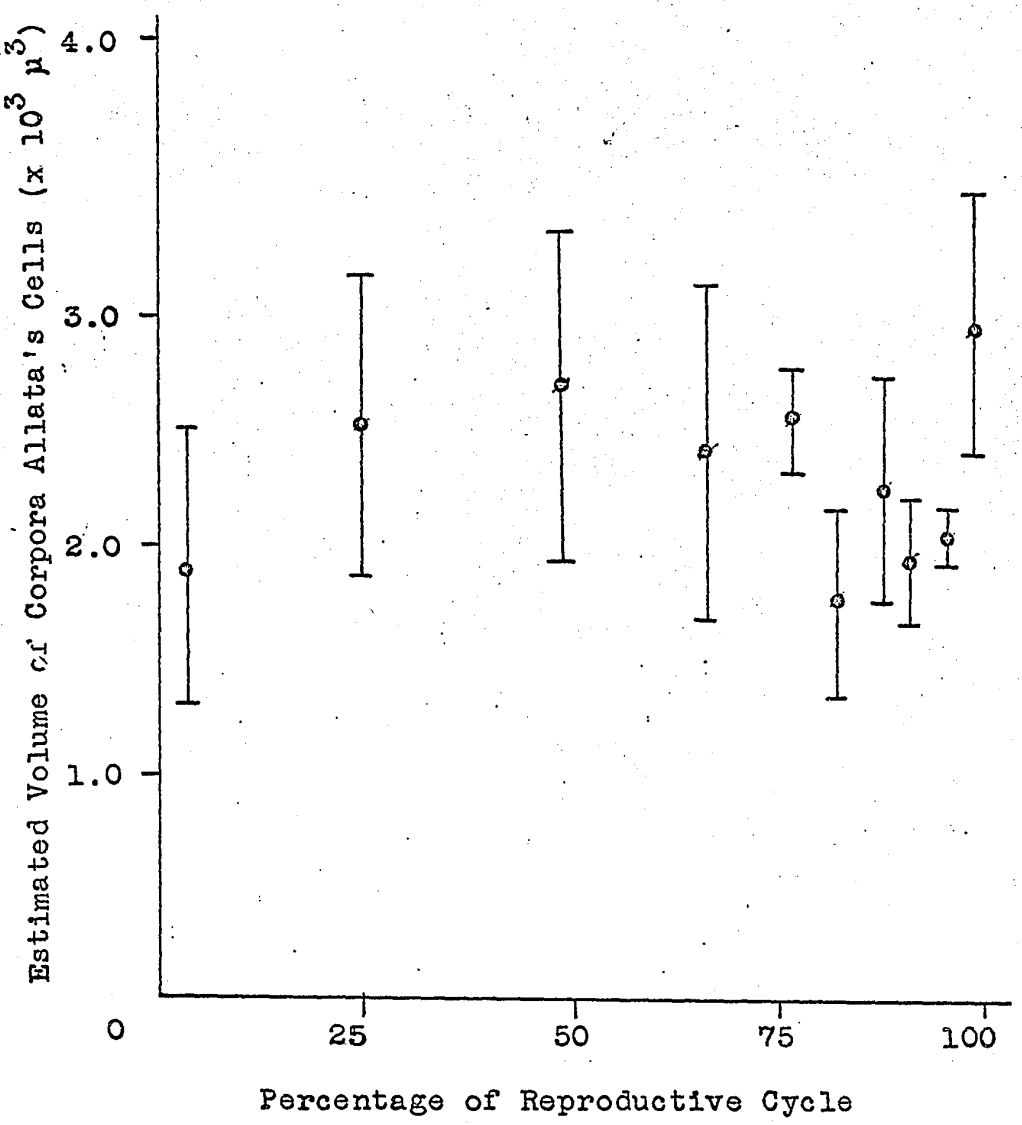


Figure 26. Graph showing the variations in the estimated volumes of the cells of the corpora allata with the reproductive cycle of the ked. Note the large variations.

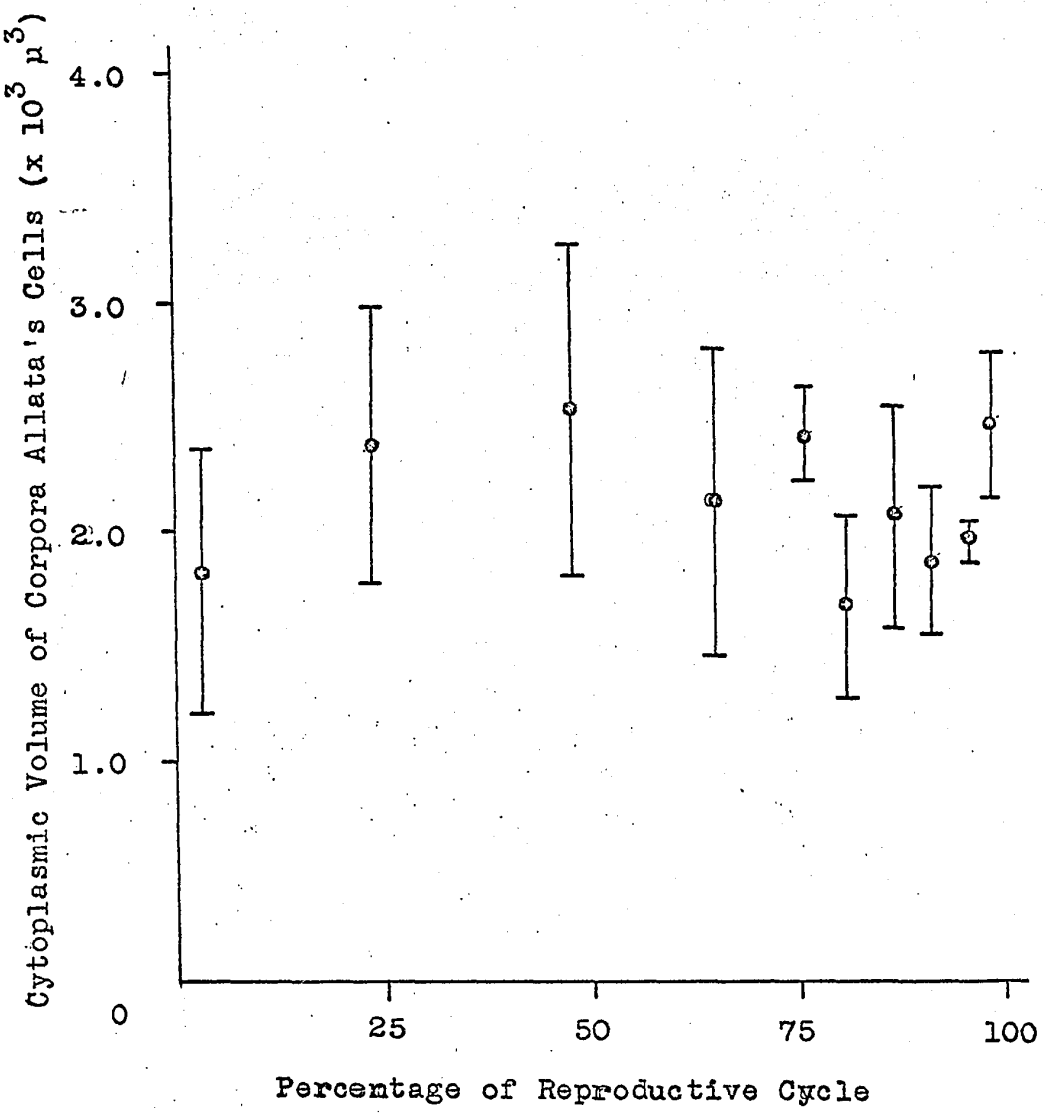


Figure 27. Graph showing the variations in the estimated cytoplasmic volumes of the cells of the corpora allata with the reproductive cycle. Note the large variations.

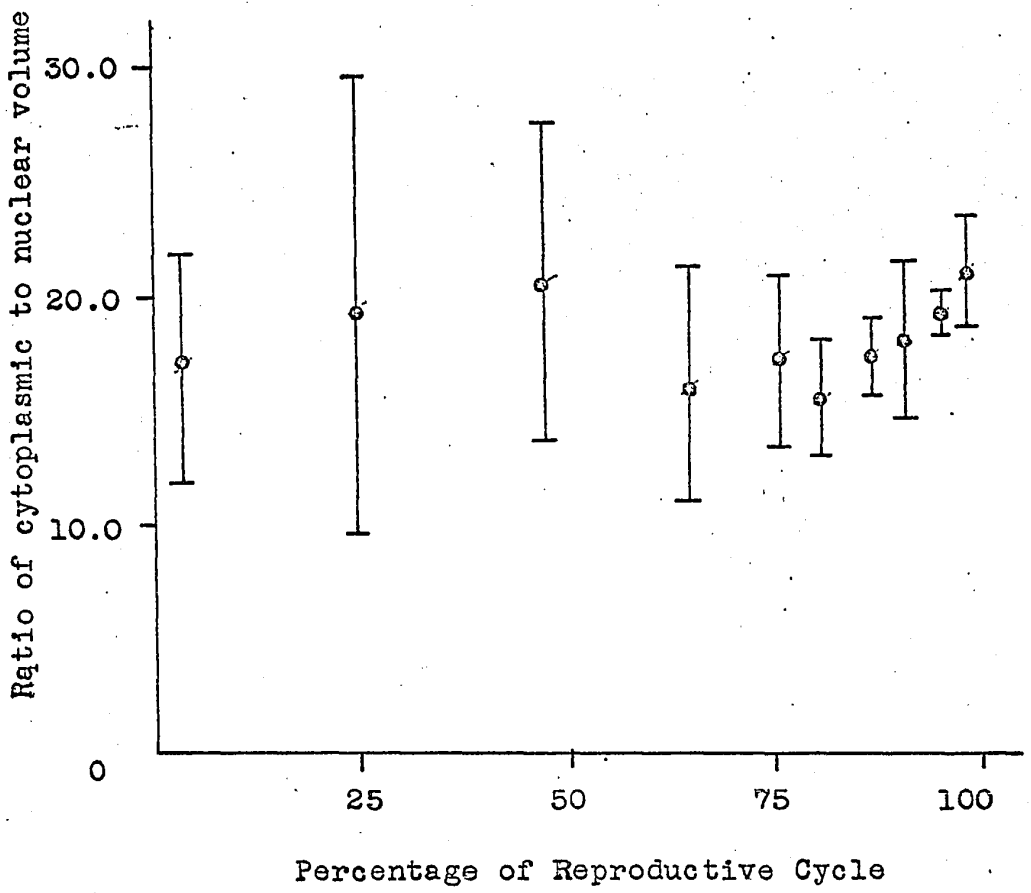


Figure 28. Graph showing the variations in the ratio of cytoplasmic to nuclear volumes of the cells of the corpora allata with the reproductive cycle of the ked. Note the large variations.

CHAPTER VI

DISCUSSION OF RESULTS

From the results of this investigation described in the previous two chapters, it is clear that the retrocerebral system of Melophagus is, in many ways, unique. In this chapter, some of these peculiarities will be discussed in the light of what is known about insect endocrines.

One peculiarity of the ked is the apparent lack of neurosecretory cells in the pars intercerebralis. It must be pointed out that the existence of these cells in the ked has not been disproven. Secretion and release may be continuous so there is no accumulation of neurosecretory material at any time or, alternatively, neurosecretory material may be present which is not detectable by the techniques used.

Langley (1967a, 1967b) has shown that in the tsetse fly, Glossina morsitans, secretion from the medial neurosecretory cells may leave these cells devoid of stainable neurosecretory material. However, this is

not true of all flies examined (Langley, 1965) as it is in the ked. In addition, there are no apparent cerebral neurosecretory cells in ked larvae or pupae. It seems reasonable that at some stage in the insect's life cycle there would be a detectable amount of neurosecretory material present in the nerve cells of the pars intercerebralis if these are, in fact, neurosecretory.

These same comments apply to the lack of apparent secretion in the nerve tracts of the brain, in the NCC, and, especially, in the corpus cardiacum. Even if cerebral neurosecretory cells secrete continuously, one might expect to find some accumulation of neurosecretory material in one or all of these places. This is not the case.

There exist, of course, neurosecretory cells that do not stain purple with PAF. The lateral neurosecretory cells of the pars intercerebralis stain with orange G in Halmi's mixture, the counterstain used in the PAF technique. Some neurosecretory cells in the thoracic ganglia of some insects are azan positive and PAF and CAHP negative (de Bessé, 1967).

However, Arvy and Gabe (1962), in their study of the histochemistry of the medial neurosecretory cells,

showed that these cells, in all insects examined (15 species), are PAF positive. It seems more likely that there are no medial neurosecretory cells at all in the ked than that they are present and are PAF negative.

To this argument must be added the fact that neurosecretory cells are not detected by the other stains used. It would be interesting to examine the pars intercerebralis under the electron microscope to see whether membrane bound vesicles characteristic of neurosecretory systems (see above, p. 15) are also absent.

Ulrich (1963), in his study of Hippoboscan endocrine systems, found medial neurosecretory cells in Ornithomyia, Stenepteryx, and Crataerina and lateral neurosecretory cells in Ornithomyia. He does not describe these cells in Melophagus; however, he does describe neurosecretory cells in the suboesophageal ganglion of some newly emerged female keds. I examined the suboesophageal ganglion of four such newly emerged female keds stained with PAF and failed to find evidence of these neurosecretory cells.

The lack of a recurrent nerve in the ked is surprising; however, Ulrich (1963) also failed to find one here, even using the vital dye methylene blue,

which is often used to detect nerves (Pantin, 1964). The lack of a frontal ganglion is less surprising. Pratt (1900) claims that the frontal ganglion rudiment in Melophagus disappears early in the larval development of the insect. Langley (1965) suggests that this lack may be a general feature of the Cyclorrhapha.

There is a single pair of nervi corporis cardiaci (NCC) in the ked. This is in accord with the situation in the Diptera in general (Cazal, 1948). Though not provable from the results of this investigation, the origin of the NCC is probably the nerve cells of the pars intercerebralis. As this is the case in other insects (p. 17), I feel it is a fair assumption that it is also so in Melophagus.

The corpus cardiacum of the ked is an interesting structure. Day (1943b) describes it as being fused with the hypocerebral ganglion and very degenerate, in fact almost absent in the adult.

I am at a loss to explain these observations. The only sign of so-called "degeneration" I have noted is the fact that the cardiacum is a loosely knit organ. Perhaps Day confuses the intrinsic cells (Figs. 20-23)

with large nerve cells of the hypocerebral ganglion and the small cells (below) (Figs. 21-23) with intrinsic cells; his figures would lead one to suspect this.

As mentioned above (p. 40) the large cells appear to be quite active secretory cells. The rest of the cardiacum is somewhat confusing. There is a network of nerve fibres and some small cells. These small cells may be glial cells associated with the nerve fibres or they represent neurons of the hypocerebral ganglion. The small size of these cells and the lack of a recurrent nerve in the ked make this latter possibility seem somewhat unlikely but do no not entirely rule it out.

Apart from the fact that the corpora allata of the sheep ked are paired, an uncommon situation in the Cyclorrhapha, this organ does not differ appreciably from that of other Diptera (E. Thomsen, 1942; Cazal, 1948; Langley, 1965; Burgess and Rempel, 1966). My observations on this organ confirm, for the most part, those of Day (1943b).

The corpora allata of the sheep ked appear to be almost continuously active. This activity cannot be correlated with events of the reproductive cycle.

This lack of an apparent cycle of allatal activity does not eliminate the possibility that such a cycle exists. It is possible that, although each ked's allata undergo cycles of secretion, those of different keds differ from each other, for instance, in the phase of the cycle. This type of situation is illustrated diagrammatically in Figure 29. In this case, there is considerable variation in the activity of different allata at any one time.

However, in most other insects studied, the allata increase in activity prior to vitellogenesis and decrease afterwards. This is the case, for instance, in the blowfly Calliphora erythrocephala (E. Thomsen, 1942) and in the viviparous cockroaches Leucophaea maderae and Diploptera punctata (Engelmann, 1968). In Leucophaea and Diploptera, in fact, the allata are inactive for most of the duration of the gestation period (see above, pp. 31-32). Why is this pattern of allatal activity not followed by Melophagus?

The answer to this question perhaps lies in the form viviparity takes in the ked. As mentioned above (p. 3), the female ked nourishes the developing larva with the proteinaceous "uterine-milk" from her milk-gland

(Pratt, 1899). This does not occur in Calliphora, which is not viviparous, or in Leucophaea or Diploptera, which are. Thus, there is, in the female ked, a constant need for protein metabolism, not only for vitellogenesis as in most other insects, but also for the synthesis of milk proteins. The function of the hormone of the ked's corpora allata may be to control protein metabolism.

Of interest relative to the hormonal control of reproduction in the ked is the situation in the tsetse fly, Glossina. As mentioned above (Chapter I), the form viviparity takes in the tsetse fly is remarkably similar to that in the ked. Langley (1967a, 1967b) showed that the neuroendocrine system of G. morsitans is active during the adult life span of the fly but that there is no detectable cycle of secretion and release, a situation similar to that of the ked. He gives evidence relating the activity of the neuroendocrine system (the cerebral neurosecretory cells) to protein metabolism, specifically to the synthesis of gut proteases (Langley, 1967b).

There are at least two explanations of the way in which a hormone from the ked's corpora allata might influence protein metabolism. One is to influence

protein synthesis; the other is to regulate protein uptake by various organs.

The function of the hormone of the corpora allata of the ked may be to stimulate protein synthesis, including that of gut proteases. This would explain the activity of the allata of the male as well as of the female. Blood, on which keds feed, is rich in proteins. The digestion of these proteins is, of course, of great importance. Fluctuations in allatal activity might be correlated with feeding times. In this connection, it would be interesting to starve keds for a few days and then to examine their allata. If protease synthesis is controlled by a hormone of the corpora allata, the allata of starved keds might be expected to be inactive.

The situation in female keds is more complex. They, of course, require protease synthesis for digestion; however, they also require protein synthesis to make yolk proteins and uterine milk.

Alternatively, a hormone from the corpora allata might influence protein uptake by various organs. It seems reasonable, in this case, that there would be fluctuations in allatal activity of the female keds

that correspond to fluctuations in the need for protein uptake by the milk-gland. This latter event might be mediated by the feeding of the larval ked in the uterus. Pratt (1900) observed that this event in keds is an active discontinuous process; this is unlike the viviparity of human beings in which the foetus is nourished passively and continuously through the placenta. It seems likely, though this has never been investigated, that the larval ked feeds at unpredictable times during the gestation period and, for this reason, the activity of the milk-gland fluctuates irregularly. This would explain the variations in allatal activity of the female ked.

If this explanation is, in fact, correct, one would expect the allata of male keds to be less irregular in their activity than those of female keds, as males need protein uptake only for normal growth and activity. This has not been examined in enough detail for any conclusions to be made on the subject.

It must be emphasized that the above suggestions are merely hypotheses; they make sense according to known facts, but are unsupported by any but the slimmest experimental evidence. They do, however, show a direction for further experimentation on ked endocrines to follow.

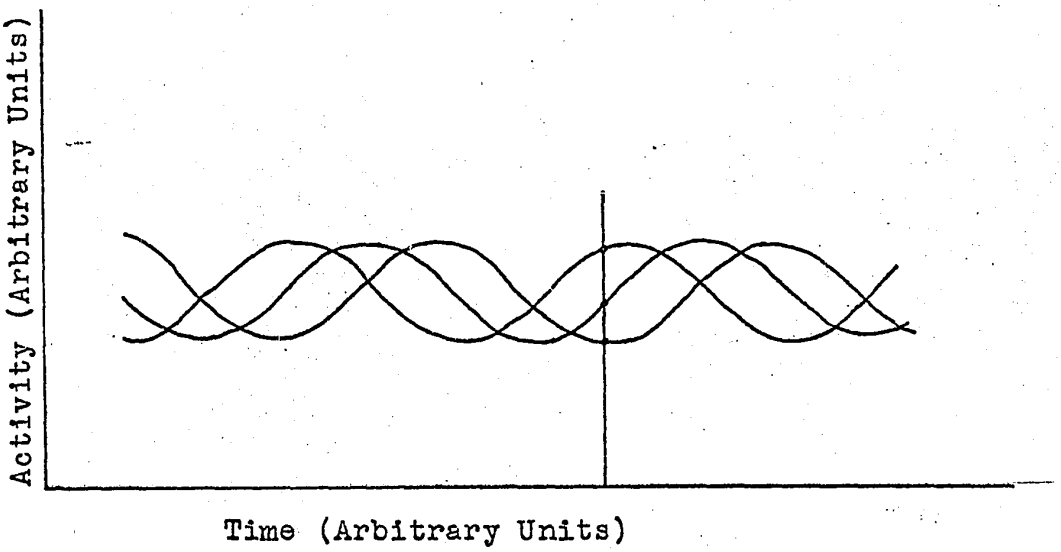


Figure 29. Graph of three hypothetical allata showing definite cycles of activity that are each one out of phase with the others. Note that at any one time (vertical line) there is a large amount of variation in activity between the different allata.

APPENDIX A

REARING AND COLLECTING KEDS

Keds were reared on several sheep kept at the Institute of Parasitology. No special arrangements were made in deference to the keds; some of the sheep were even used as sources of blood for immunology research. Most of the keds were obtained from only two of the sheep, "Clem" and "Murphy," because these two were tamer than the others.

There was no problem in keeping a large infestation during the autumn, winter, and spring. Luckily, by the time the sheep developed a resistance to the parasite (see above, p. 7) (around June, 1968), all the experimental work had been completed.

Collecting keds was done by simply patting down the wool and detaching them as they were exposed. Those used for comparative studies were kept for a few hours on a piece of wool in the laboratory for the effects of the stress to wear off. This stress did not appear to have an adverse effect on them.

APPENDIX B

TECHNIQUES USED IN THE MORPHOLOGICAL STUDIES

The morphology of the ked retrocerebral system was studied from two approaches: by dissections and by histological methods.

1. Dissection techniques

Dissections were carried out on live keds in 1.1% NaCl and on keds fixed previously in a modified Bouin's fluid (see below, pp. 81-82) and stored in 70% alcohol. Most were performed on the fixed material.

Live keds were immobilized for dissections by exposure to chloroform fumes for a few seconds. They were then placed in a wax-bottomed Petri dissecting dish and a small area of the wax was melted with a hot probe. The ked was placed in the desired orientation in the molten wax and the wax was then allowed to cool and harden. The dish was then filled with 1.1% NaCl under which the dissection took place.

Fixed keds were handled similarly except that

the abdomen, if it had not been completely removed at fixation (see below, p. 82) and the legs were generally cut away and the remaining head-thorax dried on a piece of tissue paper. The ked was then affixed to the dissecting dish (as above), covered with 70% alcohol, and dissected.

Dissections were performed under a Zeiss dissecting microscope. Instruments used were fine jeweler's forceps (number 4), razor-blade scalpels (Pantin, 1964), and fine dissecting needles made by attaching a number 000 insect pin to a glass rod with sealing wax.

To aid in interpreting structures, fixed keds were often hydrated and stained <u>in situ</u> in Hansen's iron trioxyhaematin, Halmi's mixture, or Hubschman's aniline blue-orange G counterstain (see below, p. 85). A few unsuccessful attempts were made to render nerves visible by the vital dye methylene-blue by the method of Burgess and Rempel (1966).

2. <u>Histological techniques</u>

Histological techniques were performed exclusively on tissues fixed in either a modified alcoholic Bouin's fluid utilizing trichloracetic acid instead of the usual

acetic acid component (Halmi, 1952; Davenport, 1960), or in Zenker's fluid (Davenport, 1960). The abdomen was routinely cut away from the rest of the body at fixation as this was found to be necessary for adequate penetration of the fixative.

Bouin's fixed tissues, after being kept in the fixative for 24 to 72 hours, were transferred to and stored in 70% alcohol. Those in Zenker's were fixed for four to eight hours and washed in running water overnight, after which they were gradually dehydrated to 70% alcohol where they were stored.

Keds were either processed whole and sectioned with tape (below) or the brain, thoracic ganglion, and surrounding tissues were dissected out and processed.

Whole keds were dehydrated in ethyl alcohol, cleared in benzene, and embedded in paraffin in a vacuum oven. Sectioning was done on a Cambridge rocking microtome at thicknesses of from 8 u to 12 u. A piece of cellulose tape was placed over the paraffin block to prevent crumbling of the tissues caused by the hard exoskeleton of the insect. This is the "tape method" of Palmgren (1954) and Beckel (1959).

The major problem in using tape is removing it before processing the sections. As all previous methods (Palmgren, 1954; Beckel, 1959; Feuer, 1967) were found to be unsatisfactory, I developed an easy and effective technique to accomplish this.

A microscope slide is smeared with glycerine-albumen adhesive and is then coated with liquid paraffin. Tapes are cut to the desired length and width and are floated on the liquid paraffin, tissue side down. Curling is very limited and is controlled by pressing the tape firmly to the slide. When all the required ribbons of tape are attached, a second microscope slide is placed over the tapes to keep them flat and the slides are kept overnight on a slide warming tray.

The slides are now stored until needed.

When processing the slides, the tape's adhesive may be dissolved in chloroform leaving the tissues ready to be stained. Dewaxing, hydration, and staining may now be carried out in the usual way.

This technique worked very well, but since the tape method is rather awkward and time consuming, its use was confined to the preliminary stages of this

investigation. It was found to be much easier to dissect the desired tissues from keds and to process them, rather than to work with the whole animal and use tape.

Dissections to remove the brain, thoracic ganglion, and surrounding tissues were performed with the ked's dorsal side exposed. The thoracic and most of the head cuticle was cut away leaving a collar of cuticle in the neck region. A sharp scalpel was inserted under this and it was carefully removed. This procedure exposed the desired tissues.

Scalpel cuts then separated these tissues from those surrounding them and the brain-thoracic ganglion complex could be gently teased out. Some cuticle generally remained around the neck, but this was found to be inconsequential. With practice, this dissection could be carried out successfully in nearly 100% of the attempts.

Dissected Bouin's fixed tissues were kept for another 24 hours or more in fresh 70% alcohol to wash out residual picric acid.

Before embedding dissected tissues, they were placed in a dilute solution of eosin in 70% alcohol

for 15 to 20 minutes; this rendered them a red colour which was easy to see during subsequent processing. This step was made necessary by the small size of the dissected tissues.

Dehydration, clearing, and embedding were done as described above (p. 82) for whole keds. Sectioning was done on a Cambridge rocking microtome at thicknesses of from 5 μ to 12 μ, the usual being at 8 μ; some thin and thick sections were cut at 3 μ and at 25 μ.

Paraldehyde fuchsin (PAF) after permanganate oxidation, a stain adapted for the study of neurosecretion, was the most common used. Ewen's (1962) preparation of the stain with Cameron and Steele's (1959) use of Halmi's (1952) mixture as a counterstain was used. The technique was also performed once without prior permanganate oxidation. A brain of Rhodnius prolixus (Hemiptera) was run simultaneously to check the reaction of the stain to known neurosecretory cells. This stain was also used in situ by the method of Dogra and Tanden (1964).

Two azocarmine (azan) methods were used: that of Heidenhein (Pantin, 1964) and that of Hubschman (1962). Hansen's iron trioxyhaematin method (Pantin, 1964) was

also used, both alone and with the aniline blue-orange G counterstain of Hubschman's azan technique.

These three stains - PAF, azan, and Hansen's - were the most commonly used in this investigation. Other stains tried were Gomori's chrome-alum-haematoxilin-phloxine (CAHP) (Humason, 1962), Masson's trichrome (Pantin, 1964), and Mann's methyl blue-eosin (Pantin, 1964). None of these latter three techniques contributed much to the study.

APPENDIX C

CALCULATION OF ALLATUM MEASUREMENTS

The quantitative measurements used in estimating the activity of the corpora allata are best described by the use of an example. I will describe ked E15, a female ked with a larva 1.23 mm. in length in her uterus. The brain and thoracic ganglion were dissected from her, sectioned at 8 μ and stained with PAF.

1. Corpus allatum volume

The volumes of the corpora allata were found by a simple, though indirect, technique. Images of each section of the gland were projected onto a piece of typewriter paper from a projecting microscope. They were drawn onto this paper, and the drawings were cut out and weighed. Weights obtained in this case for the two allata were 0.096 gm. and 0.101 gm.

The paper was "calibrated" by projecting, at this same magnification, the image of a stage micrometer onto a piece of graph paper. A known distance on the graph paper could, in this way, be seen to correspond

to a given length on the stage micrometer. A square was then drawn on the graph paper with sides corresponding to this known length; this square was transposed onto the typewriter paper by redrawing it over a piece of carbon paper. The square of white paper was cut out and weighed and the volume it represented was calculated on the basis of the 8 μ thick sections. The final figure obtained was found to be 1.59×10^6 μ^3/gm.

Thus, the volumes of the two allata were calculated to be 1.52×10^5 μ^3 and 1.60×10^5 μ^3; the total allatal volume was 3.12×10^5 μ^3.

2. Estimated allatum cell volume

The estimated volumes of the cells of the corpora allata were calculated by dividing the number of cells in both allata into the total volume of the two allata:

$$V_{cell} = \frac{V_{allata}}{N} \qquad (1)$$

where:

V_{cell} = Estimated cell volume

V_{allata} = Total volume of the allata

N = Number of cells in the allata

The volume of the allata was found as in section 1 of this Appendix. The value of N was found by using Floderus' (1944) formula as cited by Marrable (1962):

$$N = \left(\frac{T}{T + D - 2k} \right) n \qquad (2)$$

where:

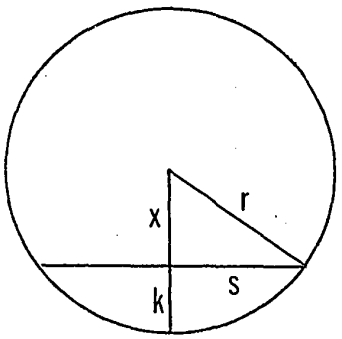
T = Thickness of the sections

D = Average nuclear diameter

k = Thickness of the smallest visible nuclear fragment

n = Observed number of nuclear fragments

The value of "T" was 8 μ for all keds used in the study of the endocrine control of ked reproduction. Nuclear diameters were measured with an ocular micrometer and averaged to find "D". In ked E15, "D" was found to equal 5.4 μ. The smallest visible nuclear fragment was 4.3 μ. The value of "k" was calculated:



r = radius of nucleus. 2.70 μ

s = $\frac{1}{2}$ smallest visible fragment. 2.15 μ

So, by the Pythagorean theorem:

$$r^2 = x^2 + s^2$$

$$x = \sqrt{r^2 - s^2}$$

$$= \sqrt{2.29\ \mu^2 - 4.65\ \mu^2}$$

$$x = 1.62\ \mu$$

$$k = r - x$$

$$= 2.70\ \mu - 1.62\ \mu$$

$$k = 1.08\ \mu$$

The value of "n" was found by simply counting all visible nuclear fragments in each allatum. Here it was found to equal 64 and 69 respectively for the two allata.

So, from formula (2)

$$N_1 = \left(\frac{8}{8 + 5.4 - 2(1.08)} \right) 64$$

$$= 45$$

$$N_2 = \left(\frac{8}{8 + 5.4 - 2(1.08)} \right) 69$$

$$= 49$$

The total number of cells in the two allata is $45 + 49 = 94$.

The estimated volume of allatal cells may be calculated from formula (1):

$$V_{cell} = \frac{3.12 \times 10^5 \ \mu^3}{94 \text{ cells}}$$

$$= 3.32 \times 10^3 \ \mu^3/\text{cell}$$

3. Cytoplasmic volume

One can, knowing the average diameter of the allatal cells' nuclei, calculate the average volume of these nuclei, assuming the nuclei to be spherical (an assumption which is not strictly true but, as comparative rather than absolute values are important, is permissible. The same might be said of my calculation of "k" in the previous section).

The formula for volume of a sphere is:

$$V = \frac{4}{3} \pi r^3$$

where:

V = volume

r = radius

In ked E15, "r" was found to be 2.70 μ. "V" thus equals 80 μ^3.

The cytoplasmic volume can be found as follows:

$$V_{ctpl} = V_{cell} - V_{nuc}$$

where:

V_{ctpl} = Cytoplasmic volume

V_{cell} = Cellular volume

V_{nuc} = Nuclear volume

so:

$$V_{ctpl} = 3.32 \times 10^3\ \mu^3 - 0.08 \times 10^3\ \mu^3$$

$$= 3.24 \times 10^3 \mu^3$$

4. Ratio of cytoplasmic to nuclear volume

This is calculated straightforwardly:

$$\frac{\text{Cytoplasm}}{\text{Nucleus}} = \frac{3.24 \times 10^3\ \mu^3}{80\ \mu^3}$$

$$= 40.5$$

APPENDIX D

STATISTICAL METHODS

The statistical tests used in this study were the analysis of variance and the Mann-Whitney U-test. Here, as in the previous appendix, I feel that the best way of explaining my methods is to cite examples.

1. Analysis of Variance

The analysis of variance was performed on the data used in the quantitative estimation of allatal activity; i.e., the allatal volume, the estimated allatal cell volume, the cytoplasmic volume of allatal cells, and the ratio of cytoplasmic to nuclear volumes of the allatal cells. This test compares the groups (A, B, C, etc.) within the total population and examines whether the variance between the groups is greater than or less than that within each group. The test is in the form of an "F" test. A statistically significant result (here at the 0.05 level) implies that there are real differences between the groups; lack of significance implies that all the groups are from the same

universe or that the variance within the groups is too great for meaningful comparisons to be made.

The technique used was the short method of Moroney (1958). As an example, I will examine the data of estimated cellular volume of the allatal cells.

The data are arranged in tables 4 and 5. The sums of all values in table 4 (the sum of column 3 in table 5) (T) is 140.79; the total number of observations (N) is 61. These two figures are used to calculate a "correction factor" (C.F.):

$$C.F. = \frac{T^2}{N}$$

$$= \frac{(140.79)^2}{61}$$

$$C.F. = 325$$

The "total sum-of-squares" is calculated by squaring each observation in table 4, summing these values, and subtracting the correction factor. The sum of these squares (table 5, column 4) is 349.64; thus, the total sum-of-squares" is 350 - 325 = 25.

The "between-groups sum-of-squares" is calculated by squaring the sums of the observations of each group (table 5, column5), dividing this by the number

of observations in that group (table 5, column 6), summing these, and subtracting the correction factor. This is 331 - 325 = 6.

The "within-groups sum-of-squares" equals the total sum-of-squares minus the between-groups sum-of-squares; i.e., 25 - 6 = 19.

The degrees of freedom (d.f.) for the total sample is the total number of observations minus one (61 - 1 = 60). The between-groups d.f. equals the number of groups minus one (10 - 1 = 9). The within-groups d.f. equals the total d.f. minus the between-groups d.f. (60 - 9 = 51).

Variances may now be computed. These are equal to the sum-of-squares divided by the degrees of freedom. So, the between-groups variance is 6/9 = 0.67; the within-groups variance is 19/51 = 0.37. With this, one can calculate:

$$F = \frac{\text{Variance between-groups}}{\text{Variance within-groups}}$$

$$= \frac{0.67}{0.37}$$

$$F = 1.81$$

Entering the F table with 6 d.f. as that of the greater variance and 51 d.f. as that of the lesser, one finds that the probability is greater than 0.05 that the differences between the individual groups is not real; this is not significant. As discussed above on page 92, this implies either that the data are all of the same universe or that the data for each group are too variable for any meaningful conclusions to be made. This latter conclusion, I feel, represents the true situation.

2. The Mann-Whitney U-test

The U-test is used to compare two populations and determine whether they are significantly different from each other. A more usual test of this is the t-test; however, as discussed by Siegel (1956), the U-test is much more reliable for this purpose. My procedure is after Siegel (1956). As an example, I will compare the corpora allata volumes between groups F and H.

There are two values of U; the formulae are:

$$U = n_1 n_2 + \frac{n_1 (n_1 + 1)}{2} - R_1 \qquad (1)$$

and

$$U = n_1 n_2 + \frac{n_2 (n_2 + 1)}{2} - R_2 \qquad (2)$$

where:

n_1 = Number of observations in the smaller group

n_2 = Number of observations in the larger group

R_1, R_2 = Sum of ranks in the smaller and the larger groups (see below).

The value of U used in this test is the smaller. It is related to the other (U') by this equation:

$$U = n_1 n_2 - U' \qquad (3)$$

To find R_1 and R_2, one first arranges the data in order of magnitude. In this case:

Group F: 125×10^3; 134×10^3; 165×10^3; 224×10^3

Group H: 159×10^3; 176×10^3; 246×10^3; 267×10^3; 269×10^3

As group F is the smaller one, $n_1 = 4$ and $n_2 = 5$.

Ranks are now assigned to the data, the rank 1 for the smallest value, 2 for the next largest, etc. So:

Group F: 1, 2, 4, 6

Group H: 3, 5, 7, 8, 9

These ranks are summed to determine R_1 and R_2:

$R_1 = 13$

$R_2 = 32$

Substituting these figures into formulae 1 and 2, one finds $U = 3$, and $U' = 17$. These figures may be confirmed by substituting U' into formula 3.

A table of values of U, such as is given in Siegel (1956), is now entered with $n_2 = 5$, and $n_1 = 4$, and $U = 3$. The probability that the two groups are different is 0.056. This is nearly, but not quite, significant at the 0.05 level. Thus, the two groups are not significantly different from each other.

TABLE 4. Measurements of cellular volume of the corpora allata, for analysis of variance ($\times 10^3\ \mu^3$)

Group A	Group B	Group C	Group D	Group E
1.82	2.38	2.20	1.58	2.24
3.00	3.39	3.98	3.04	2.86
1.76	1.73	1.31	2.32	2.51
2.26	2.96	1.72	1.97	2.49
1.00	2.14	2.45	2.90	2.52
1.84	2.57	2.86	3.74	12.62
1.52	2.51	2.85	1.49	n = 5
13.20	3.32	3.27	2.81	
n = 7	2.38	2.94	1.64	
	2.84	2.85	21.49	
	1.21	26.43	n = 9	
	27.43	n =10		
	n =11			

Group F	Group G	Group H	Group I	Group K
1.84	1.65	1.97	2.15	2.50
1.12	3.07	1.46	1.93	2.19
2.09	2.25	3.24	4.08	3.03
1.97	2.28	1.85	n = 2	7.72
7.02	1.83	2.20		n = 3
n = 4	11.08	9.72		
	n = 5	n = 5		

TABLE 5. Data for calculation of analysis of variance. Cell volume.

(1)	(2)	(3)	(4)	(5)	(6)
A	7	13.20	27.22	174	25.0
B	11	27.43	72.59	753	68.4
C	10	26.43	75.11	700	70.5
D	9	21.49	56.21	462	51.3
E	5	12.62	32.05	159	31.8
F	4	7.02	12.89	49.3	12.3
G	5	11.08	25.71	123	24.6
H	5	9.72	19.29	94.5	18.9
I	2	4.08	8.34	16.9	8.5
K	3	7.72	20.23	59.6	19.9
Totals	61	140.79	349.64		330.7

(1) Group
(2) Number (n) of observations in that group. The sum of column (2) is N of p. 93.
(3) The sum of the observations in that group. The sum of column (3) is T of p.93.
(4) The sum of the square of each observation in that group. The sum of column (4) is used to calculate the "total sum-of-squares" on p. 93.
(5) The square of the items in column (3)
(6) The items in column (5) divided by the items in column (2). The sum of column (6) is used to calculate the "between-groups sum-of-squares on p. 94.

APPENDIX E

A CURIOUS STRUCTURE IN THE NECK OF THE KED

In the preliminary course of this investigation, a curious and hitherto undescribed structure was discovered surrounding the ventral nerve cord in the neck region. It was first noticed in a saggital section cut by the tape method and stained with PAF. It had the appearance of purple blotches (resembling neurosecretory material) in an expanded region of connective tissue surrounding the ventral nerve cord. It was tentatively suggested that this might be a neurohaemal organ of yet undiscovered neurosecretory cells.

Later investigations failed to reveal any neurosecretory cells in the ked and the problem was reinvestigated. In transverse sections, the PAF positive regions were seen to consist of coarse fibrils running transversely above and below the nerve cord (Figs. 12, 30). The colour of these fibrils was seen, with all stains used, to be identical to that of tendons and a few sections were obtained of muscles attaching by tendons to this region (Fig. 31). Thus, the neurohaemal organ

concept was abandoned and the idea was advanced that this is an area of muscle attachment.

The structure arises as a great expansion of the connective tissue sheath surrounding the ventral nerve cord in the neck region. It is cellular and is closely associated with cuticle and with large longitudinal tracheal trunks (Figs. 12, 30). Muscles from the thorax attach to it (Figs. 31, 34) and their tendons make up a large proportion of its bulk. Strands of cuticle have also been seen to enter it (Figs. 32, 33).

A close superficial resemblance of this structure to the vertebrate spinal column is apparent. It is a connective tissue sheath composed, in part, of the skeletal element (cuticle) of the insect, forming muscle attachments and protecting the major nerve trunk in a region of flection. Of course, I would be the last person to suggest that this structure and the vertebrate spinal cord are homologous; I merely point this out as an interesting case of parallel evolution.

Figure 30. Cross section through the structure in the neck of the ked around the ventral nerve cord. Note its make-up of fibrous tissues. Tendons can be seen running transversely (for instance, at the arrow). Zenker's, Masson's trichrome. x700.

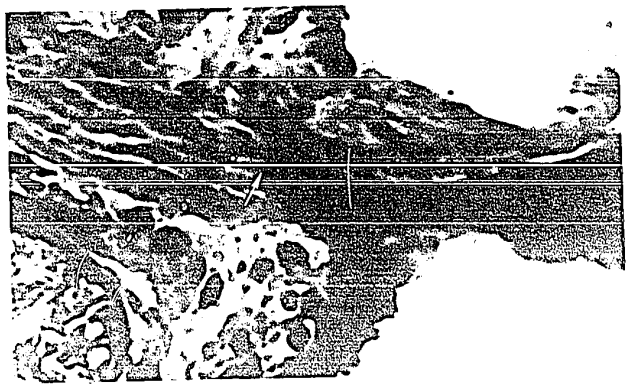


Figure 31. Enlargement of an area of Figure 30 showing a muscle attachment entering the structure in the neck around the ventral nerve cord (arrow). x1100.

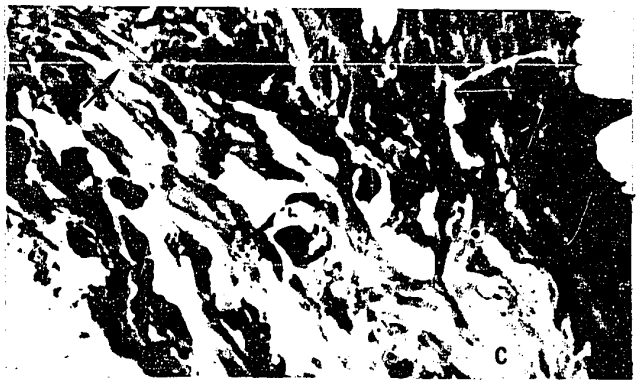


Figure 32. Section of the junction between the neck cuticle and the structure around the ventral nerve cord of the ked. Note the strands of cuticle entering this structure (arrow). Bouin's, Hansen's. x1280.

Figure 33. Same as Figure 32, a different view.

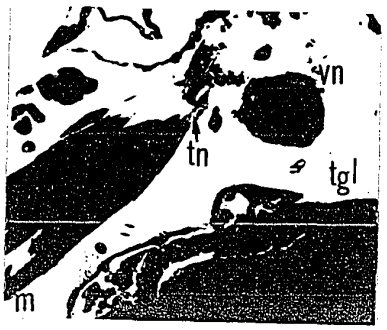


Figure 34. Oblique frontal section of the thorax of a ked, showing a muscle passing beside the thoracic ganglion. Although this section does not show its attachment, it is on the structure around the ventral nerve cord in the neck. Bouin's, Hubschman's azan. x260.

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