

Morphological and Histological Studies on the Corpora allata and cardiaca in Orthoptera

by

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Özet: 1 — Böceklerde başta ve beyin gerisinde bulunan iç salgı bezlerinden corpora allata ve corpora cardiaca'nın morfolojisi *Bolivarina brachyptera* (Pall), *Saga cappadocica* (Wern.), *Bradyporus dilatatus* (St.), *Acrida bicolor* (Thunb.), *Locusta migratoria* (L) Ph. Solitoria, *Glyphotmethis escherichi* (Kr.) türlerinde araştırılmıştır. Bundan başka corpora allata *Locusta* ve *Acrida*'da histolojik olarak incelenmiştir.

2 — Birer çift olan bu bez sistemi baş içinde, özofagusun dorsalinde ve serebral ganglionun gerisinde bulunur. Aralarında sinirlerle irtibatla bulunan bu dört bez, aynı zamanda corpora cardiaca'dan çıkan bir çift sinirle protocerebruma bağlanmıştır.

Türler arasında, bezlerin sinir sistemine ve yekdiğerine nazaran yeri bakımından bazı ufak farklar bulunmakla beraber, aynı bir familyaya dahil türlerin endokrin sistemi aralarında büyük bir benzerlik gösterir.

3 — Corpora allata'nın yapısını teşkil eden elemanlar müşahede edilen her iki türün larva ve erginlerinde tespit edilmiş ve bunlar beş grupta toplanmıştır.

a — Mezenşim hücreleri, b — Farklaşmamış hücreler, c — Normal salgı hücreleri, d — Dev salgı hücreleri (poliploid hücreler), e — Sinir hücreleri.

a — Mezenşim hücreleri: Bezin muhitini saran, uzun çekirdekli örtü hücreleridir.

b — Farklaşmamış hücreler: Sayıları pek fazla değildir. Kromatin miktarı azdır. Her larva safhasının başlangıcında kuvvetli mitoz faaliyeti görülür.

c — Normal salgı hücreleri: Bezin büyük bir kısmını bu hücreler teşkil eder. Faal olmadıkları zaman farklaşmamış hücelere benzerler. Nukleusları kromatin bakımından zengindir. Faal oldukları zaman hacimleri genişler; sitoplazmaları asidofil granüllerle dolar.

d — Dev salgı hücreleri: Sayıları bir bezde üç ilâ sekiz arasında değişir. Poliploid mitozla meydana gelirler, faaliyetleri normal salgı hücreleri gibidir.

e — *Corpora allata* siniri bez içinde bir çok kollara ayrılarak yayılır. Uzun çekirdekli sinir hücreleri kromatin bakımından zengindir.

4 — *Corpora allata*'nın faaliyeti devridir. Larva safhalarında deri değişmesinden hemen sonra bezde hiçbir faaliyet yoktur. Bir müddet sonra mitoz bölünmeleri başlar. Bundan sonra salgı hücreleri asidofil granüllerle dolar; vakuoller teşekkül eder. Boşalma faaliyeti safhanın ikinci yarısında vuku bulur. Safhanın sonunda salgı hücreleri büzülür ve bez bir miktar küçülür. Asidofil granül kesafeti çok azalmıştır. Kritik periyoda tekabül eden dördüncü larva safhasında beş ve altıncı günlerinde ifraz faaliyeti en fazladır. Son larva safhasında ise bezin faaliyeti çok kısa sürer; ifrazatı da azdır.

5 — Erginleşmeden sonra *corpora allata* cinsi olgunluğa kadar sükûnet halinde kahr. Mitoz hiçbir zaman görülmez. Dişilerde ifraz faaliyeti yumurtanın inkişafından kısa bir zaman evvel başlar ve en yüksek seviyesine çıktıktan sonra, cinsi faaliyetinin sonuna kadar bu seviyede kalır. Ergin erkeklerdeki faaliyette buna muadildir. Cinsi faaliyeti sona eren dişi ve erkeklerin *corpora allata*'sında hiçbir faaliyet görülmez.

6 — *Corpora allata*'nın salgı faaliyeti hakkında elde edilen histolojik sonuçlar, bu bez üzerinde aşağıda adları geçen yazarlar tarafından yapılmış olan fizyolojik incelemelere ve *Melanoplus*'ta elde edilen histolojik neticelere uyar ve onları teyid eder.

I — Introduction

The secretory glands of insects, the *corpora allata* and *corpora cardiaca* found in the posterior part of the head have been studied by several authors. Although many investigations have been made, the role of the *corpora cardiaca* in the life of the organism has been very little studied.

It was found by physiological studies that the *corpora allata* produced a hormone which delayed metamorphosis and brought about retention of larval characters. The hormone which is produced by this gland is called "Juvenile Hormone".

Wigglesworth, observed the marked increase in secretory activity of the cells at the critical period and concluded that this gland produced a hormone in *Rhodnius prolixus* (Hemiptera). This theory was backed experimentally by the author, in 1936.

At about the same time (1936) Bounhiol also found by experimental means that the *corpora allata* delayed methamorphosis in *Bombix mori* (Lepidoptera).

Piepho (1940) performed similar experiments on *Galleria* and obtained the same results.

Pflugfelder (1936—1938), Scharer (1946) and Thomsen (1942) in their investigations on *Dixippus morosus*, *Leucophea madera* and *Calliphora erythrocephala*, respectively, found that the corpus allatum produced a juvenile hormone.

These physiological investigations have shown that the corpora allata secrete in a cyclical pattern.

Cazal and Guerrier (1946) in Orthoptera and Cazal (1947—1948) in Odonata and some other insects studied the morphology and histology of both glands and found that the corpus allatum produced a hormone, but they could not find by physiological research exactly how this secretory activity occurred.

Mendes (1948) was the first author to relate the results of physiological experiments to histological researches.

The present study was carried out as a second part of Mendes' investigations. The secretion of the corpora allata was studied histologically in many larval instars and adults. The result obtained from these investigations were compared with the results from physiological investigations on other species of insects.

As a preliminary to this investigation the morphology of both glands was studied in six species of Orthoptera and it was shown how different these glands could be in various insect families. Other authors have shown that these glands can be similar to one another in various species of one family.

II — Material and method

All of the species of Orthoptera used in the experiments were collected from the suburb of Ankara, except *Locusta migratoria* (L) Ph. Solitaria which was brought from Diyarbakır and cultured in our department.

The species of Orthoptera on which the experiments were performed are following:

<i>Bolivaria brachyptera</i> (Pall.)	Mantidae
<i>Saga cappadocica</i> (Wern.)	Tettigoniidae
<i>Bradyporus dilatatus</i> (St.)	Tettigoniidae
<i>Acrida bicolor</i> (Thunb.)	Acrididae
<i>Locusta migratoria</i> (L) Ph. Solitaria	Acrididae
<i>Glyptmethis escherichi</i> (Kr.)	Acrididae

Among these species histological studies were made only on *Locusta* and *Acrida*. Other species were studied morphologically.

The structure of the glands and their location in the head were investigated by means of serial sections. Both *Locusta* and *Acrida* species were reared in cages from the first instar in the laboratory. In this way it was possible to be sure of the instar (and the age within each instar) of any individual examined. The following fixatives were used: Susa, Zenker, Bouin. Of these, Bouin solution was used most often, because it gave the best results.

The cuticle around the head was either separated with a fine pair of scissors before it was fixed, or the nymphs were put in the butylique alcohol to soften the skin. The embedded glands were sectioned six or seven microns thick.

The sections were stained with one of following dyes: Hematoxylin-eosin, hematoxylin-erythrosin and Masson's trichrome. Among these the hematoxylin-eosin was most commonly employed.

III — Morphology of the glands

The corpora allata and corpora cardiaca which are inserted deeply in the posterior part of the head of Orthoptera, that is at the border of the prothorax and the head, can be seen easily. These inner secretory glands, located behind the cerebral ganglion, on the oesophagus, are paired. The corpora cardiaca, which are close to one another, are directly above the oesophagus and parallel to it. The corpora allata are connected to the corpus cardiacum and situated dorso-laterally on the oesophagus (Fig. 1).

The corpora cardiaca are located close together, connected to one another by a thin and transparent tissue. This connection can be seen very clearly in cross sections (Fig. 1). The glands are symmetrical.

These two pairs of glands are connected also to the brain via nerves which come to the corpora cardiaca from the cerebral ganglion. The aorta is surrounded by the inner surfaces of corpora cardiaca (Fig. 1).

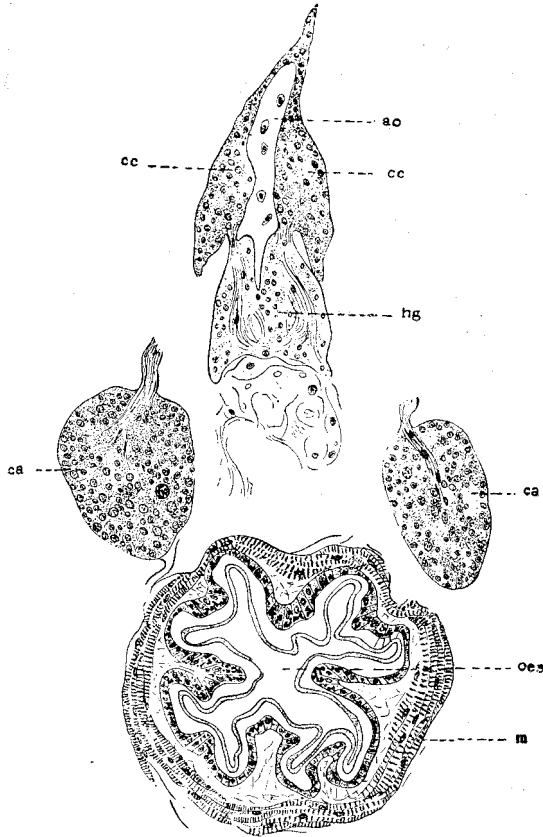


Figure 1. Cross section through corpora cardiaca, corpora allata and oesophagus of fifth nymphal instar ♀ *Locusta*. ao, aorta; cc, corpora cardiaca; hg hypocerebral ganglion, ca corpora allata; oes, oesophagus; m, muscle.

In general the corpus cardiacum is ellipsoidal and somewhat long, the corpus allatum is spherical and smaller. These glands show differences as far as their colours are concerned: The corpus cardiacum is milky white and bluish, almost non transparent. The corpus allatum is yellow - white and semitransparent. Both glands are made up of quite loose tissues.

The glands also differ in size. In general the corpora cardiaca are three times larger than the corpora allata. The drawing (Fig. 2) indicates the differences in sizes of this system of glands in various species.

The location of the hypocerebral ganglion, which is close to the corpora cardiaca and connected to them, varies somewhat in different species. The morphology and the location of the corpora allata and corpora cardiaca in relation to the nervous system differs in various species of one Grasshopper family. On the other hand these differences among other families are more apparent (Fig. 3).

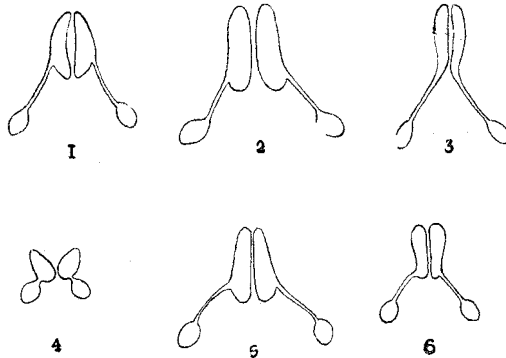


Figure 2. Diagrams of the corpora allata and cardiaca in six species of Orthoptera.

1. *Saga cappadocica* (Wern), 2. *Bradyporus dilatatus* (St), 3. *Glyptomethis escherichi* (Kr.), 4. *Bolivaria brachyptera* (Pall.), 5. *Locusta migratoria* (L.) Ph. Solitaria, 6. *Acrida bicolor* (Thunb).

These results, when considered together with morphological studies of other Orthoptera species carried out by Cazal and Guerrier (1946), bring out the following conclusion:

1 — Tettigoniidae. The glands of *Saga* and *Bradyporus*, examined in this study, and the gland systems of *Tettigonia* investigated by Cazal and Guerrier, are quite alike. In all three species the corpora cardiaca are about three times larger than corpora allata. The nerves which connect the gland pairs with one another are of considerable length. In *Tettigonia* and *Bradyporus* the hypocerebral ganglia is close to the corpora cardiaca, while in *Saga* they are posterior to these glands.

2 — Mantidae. The gland systems of *Mantis* (Cazal and Guerrier) and *Bolivaria*, which we investigated, are quite similar. In both species the gland systems are small and the

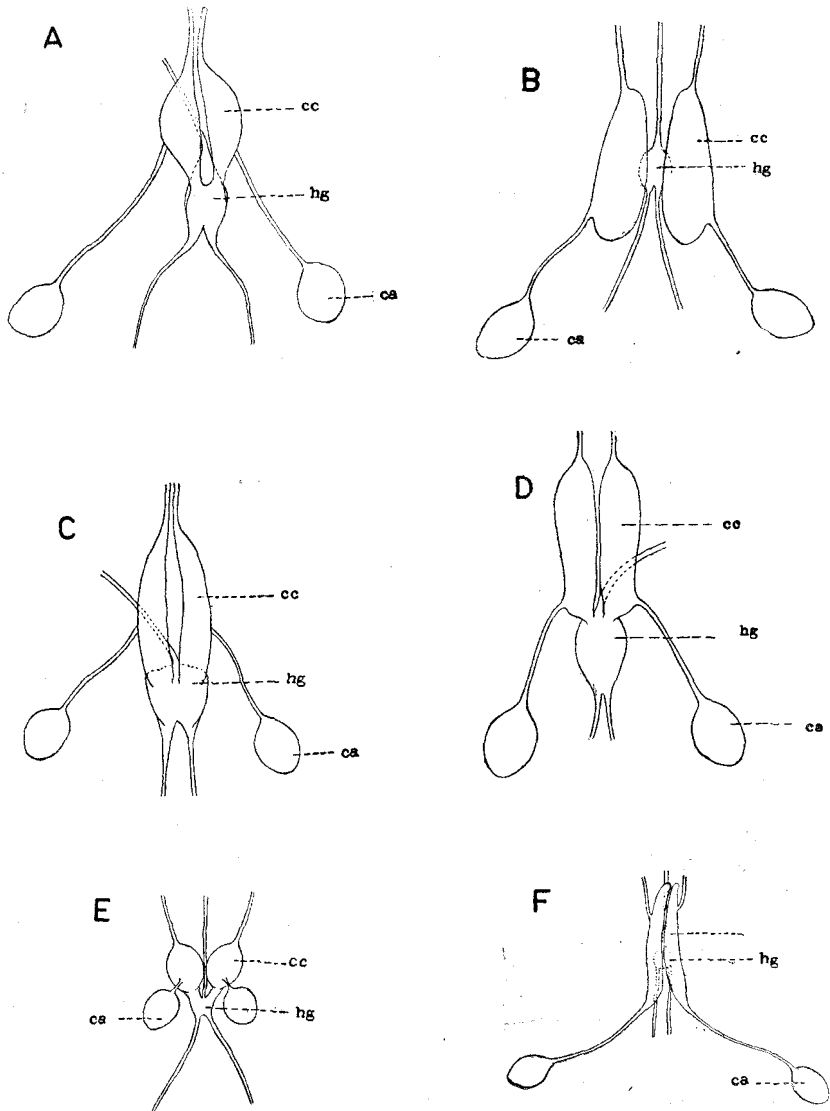


Figure 3 Morphology of the corpora allata and cardiaca in different species of Orthoptera

A - *Saga cappadocica* (Wern.), B - *Bradyporus dilatatus* (St.), C - *Locusta migratoria* (L) Ph. Solitaria, D - *Acrida bicolor* (Thunb), E - *Bolivaria brachyptera* (Pall.), F - *Glyphotmethis escherichi* (Kr.). ca, corpora allata; cc, corpora cardiaca; hg, hypocerebral ganglion.

corpora cardiaca are almost spherical. The nerve of the corpora allata is very short and therefore the two gland pairs seem to be attached to one another.

3 — Acrididae. In *Acrida* and *Locusta* and in *Orthacantacris* investigated by Cazal and Guerrier, the gland systems are of medium size. The nerve of the corpus allatum is of average length. However in the *Glyphotmethis*, which is from the same family, the connection of nerves with the corpora cardiaca is different.

In conclusion it may be said that the gland systems of different species show some small variations with regard to their general morphology and their relations to one another. There is a great similarity among the endocrin systems of the species of the same family. However, in the species which have different head formations, the location of the gland systems within the head is different.

IV — Histology of the glands

Histological investigations were made only on *Locusta migratoria* (L) Ph. Solitaria and *Acrida bicolor* (Thunb.). Nymphs and adults were studied by means of a large number of sections taken at intervals throughout the developmental stages. The structure and function of each group of cells were investigated. Each instar is illustrated.

Elements of corpora allata:

The structure of the corpus allatum is rather complicated. The elements of this gland show the same characteristics in nymphs and adults. We can group the types of cells which make up these elements in the following six groups:

a) Connective tissue cells, b) Undifferentiated cells, c) Normal secretory cells, d) Giant secretory cells (polyploid cells), e) Nerve cells, f) Tracheae.

a) Connective tissue cells (Fig. 4, A)

These are very long cells, forming a layer on the periphery of the corpus allatum. The longest diameter of the nucleus is eleven microns. These cells are rich in chromation.

b) Undifferentiated cells (Fig. 4, B)

These are large in number and are seen in every stage. Their nuclei are mostly rounded. The nucleus is nine microns in diameter. Compared with the other cells, they are poor in chromatin. When stained with hematoxylin their nuclei appear lightly coloured. This helps to separate them from other cells. At the beginning of each nymphal instar they show a strong mitotic activity.

c) Normal secretory cells (Fig. 4, C-D)

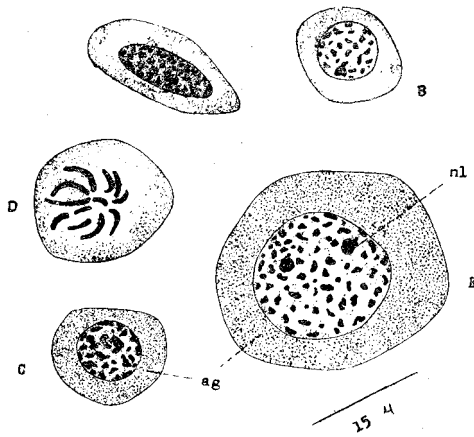


Figure 4 Types of cells in corpora allata.

- A — Connective tissue cell (adult ♀ *Acrida*).
- B — Undifferentiated cell (fifth instar ♂ *Acrida*).
- C — Normal secretory cell (fourth instar ♀ *Locusta*).
- D — Mitosis in normal secretory cell (fifth instar ♀ *Locusta*).
- E — Giant secretory cell (adult ♀ *Locusta*).

The greater part of the gland is formed by these cells and they are found in every nymphal and adult instar. The nuclei are mostly rounded or slightly ovoid. They contain a large amount of chromatin and always have a single nucleus. They are darkly stained with hematoxylin. The average diameter of the nuclei is ten microns. Immediately after moulting, it is difficult to separate these from the undifferentiated cells.

During the first few days after moulting the mitotic activity of these cells is greatly increased. Their cytoplasm is a little

acidophil. When the gland is functioning, the cytoplasm is full of acidophil granules. After a while these granules, which pile up around the nucleus, fill the whole cell.

In old instars and in adults some vacuoles with a slightly acidophil reaction can be seen. When there is enough secretion some intercellular vacuoles may appear. But these can be observed only after the secretory material is given to the blood. Generally it is difficult to distinguish cell boundaries.

Secretory cells have no specific place in the gland. They are spread throughout the gland, but at the end of each nymphal stage the nuclei of these cells can be seen in a string at the periphery of the gland. The cytoplasm and the vacuoles are generally found in the center of the cell. When the gland appears to be actively secreting and when it reaches the end of its secretory cycle, numerous fine lines may be seen following somewhat radial courses from the central regions of the gland to the periphery. They are called "Lines of flow," by Mendes (1948).

d) Giant secretory cells (polyploid cells) (Fig. 4,E)

The nuclei of these cells are three or four times as big as the nucleus of the normal secretory cell. They are nothing but polyploid cells. According to the degree of polyploidy, they contain two or four nucleoli. These giant cells can be seen during the third, fourth and fifth nymphal instars and in the adults.

Five to eight giant cells may be found in each gland of *Locusta*, rather fewer in *Acrida*.

The activity of polyploid cells is almost similar to that of normal secretory cells. They have a larger quantity of secretion than that of the secretory cells. But there is no difference in the concentration of granules in both kinds of cells. When the gland is actively secreting, some intracellular vacuoles appear in them. The giant cells are scattered at random; they can be found in the center as well as in the periphery of the gland.

e) Nerve cells

The nerve enters the gland at the anterior end and continues through it to the posterior end, sending some branches to both sides. The nuclei, which are seen along the nerve fibres, are long in shape and rich in chromatin.

f) Tracheae

There is a rich system of tracheae in corpora allata; especially among the nerve fibers, these may be seen as rounded empty cavities.

V — Secretory activity of corpora allata

A — In nymphal instars

The activity of the corpora allata was studied in some detail in *Locusta*. This activity was entirely the same as in *Acrida*, but began a little later in the latter species. The reason for this is probably that in *Acrida* the nymphal stages last longer than in *Locusta* at the same temperature.

The third nymphal instar

The third nymphal stage in *Locusta*, kept at a temperature of 27° — 29°C, lasted six to seven days.

The average dimensions of the corpora allata are as follows:

<i>Locusta</i> ,	Male:	110 μ	long	—	80 μ	wide
	Female:	120,,	„	—	85,,	„
<i>Acrida</i> ,	Male:	90,,	„	—	67,,	„
	Female:	93,,	„	—	71,,	„

The activity of the corpus allatum in *Locusta* during the six days between the two moultings is as follows:

No activity takes place the two days after moulting. In this period the secretory cells seem to be undifferentiated. Their cytoplasm contains very few secretory granules. There are no intercellular or intracellular vacuoles. On the third and fourth days, especially on the third day, the secretory cells grow in volume; their nuclei swell and prepare for mitosis. Later both secretory and undifferentiated cells undergo mitosis in large numbers. As a result of this the volume of the gland is increased.

On the fourth and fifth days following mitosis, the secretory cells are very active. At first the acidophil granules are seen around the nuclei and later they fill all of the cytoplasm.

At the end of this stage, that is, in the sixth and seventh days the secretion goes into the blood. During this period the

cells become empty. As a result of this the granules in the cytoplasm become smaller in number, but they never disappear completely.

There is almost no difference between the appearance of a gland which has completed its activity and one of an early third stage nymph. However, at the end of this instar the number of the giant cells increases and two or three polyploid cells are seen. The corpora allata in females are longer than in males. At the same time the body of the female is longer than the male. This shows that the size of the gland is proportional to the size of the body. However, this difference in size does not indicate any difference in the activity of the gland.

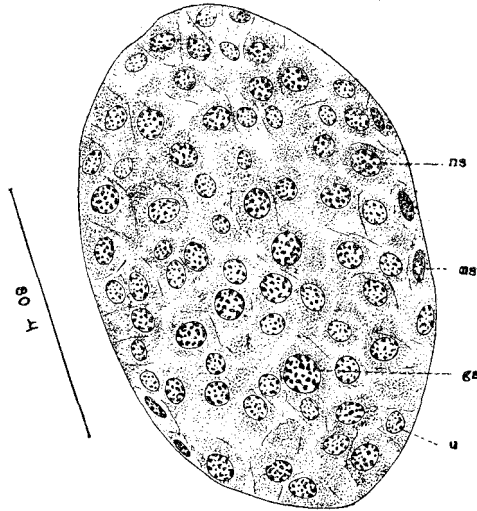


Figure 5. Cross-section through corpus allatum of fourth larval instar ♀ *Locusta*, killed one day after moulting. ns, normal secretory cell; ms, connective tissue cell; gs, giant secretory cell; u, undifferentiated cell.

Fourth nymphal instar

At the end of this stage the average dimensions of the corpora allata are as follows:

<i>Locusta</i> ,	Male:	150 μ	long	—	107 μ	wide
	Female:	180,,	"	—	155,,	"
<i>Acrida</i> ,	Male:	100,,	"	—	80,,	"
	Female:	170,,	"	—	85,,	"

The duration of the fourth nymphal stage of *Locusta* (at 27°-29°C) is about eight days. During the three days after moulting the gland does not show any activity. The cells are all similar and there are some polyploid cells in the gland (Fig. 5) The number of the acidophil granules in the secretory cells is still small.

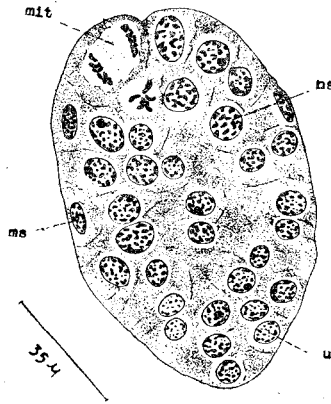


Figure 6. Cross-section through corpus allatum of fourth larval instar ♀ *Acrida*, killed 5 days after moulting ns, normal secretory cell; ms, connective tissue cell; u, undifferentiated cell, mit, mitosis.

In the following two days both secretory and undifferentiated cells show strong mitotic activity (Fig. 6). During this period new polyploid cells are produced. Although the acidophil granules in normal and giant cells are seen during every phase of this stage, the secretion is greater in fifth, sixth and seventh days. The cells are completely filled with granules; inter-cellular and intra-cellular vacuoles are formed. The corpus allatum is most active and the secretion is greatest in *Locusta* (as well as in *Acrida*) (Fig. 7,8) in the fourth instar.

At the end of the stage the empty cells are contracted after their secretory material are given to the blood. As a result of this a slight decrease in the size of the gland is seen. The number of acidophil granules is decreased as the vacuoles are emptied. The "lines of flow" extending from the center to the periphery of the gland become more clear.

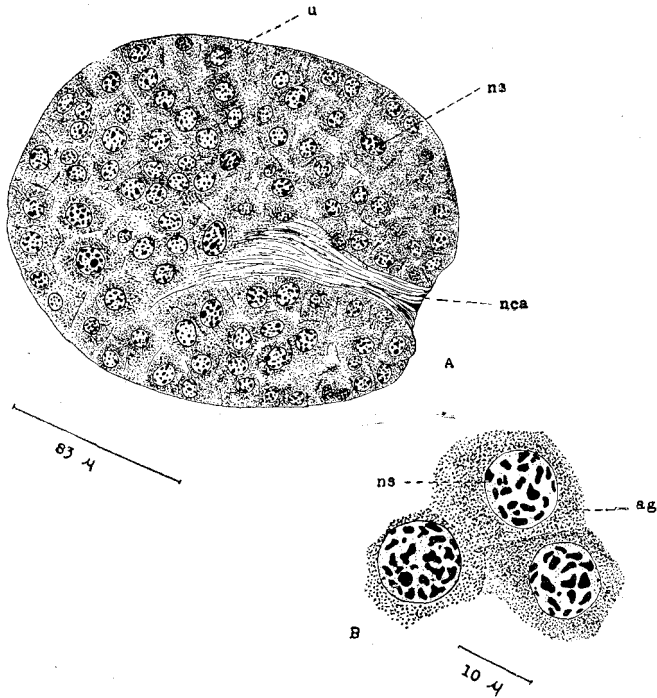


Figure 7. A-Cross-section through corpus allatum of fourth nymphal instar ♀ *Locusta*, killed 5 days after moulting. B-Normal secretory cell in the same gland. u, undifferentiated cell; ns, normal secretory cell; nca, nervus corporis allati; ag, acidophil granules.

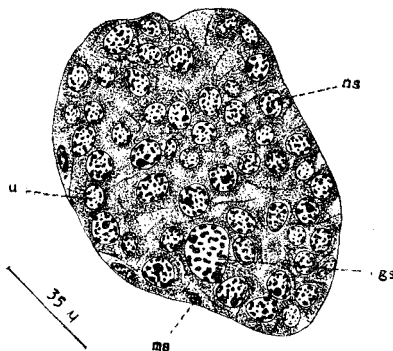
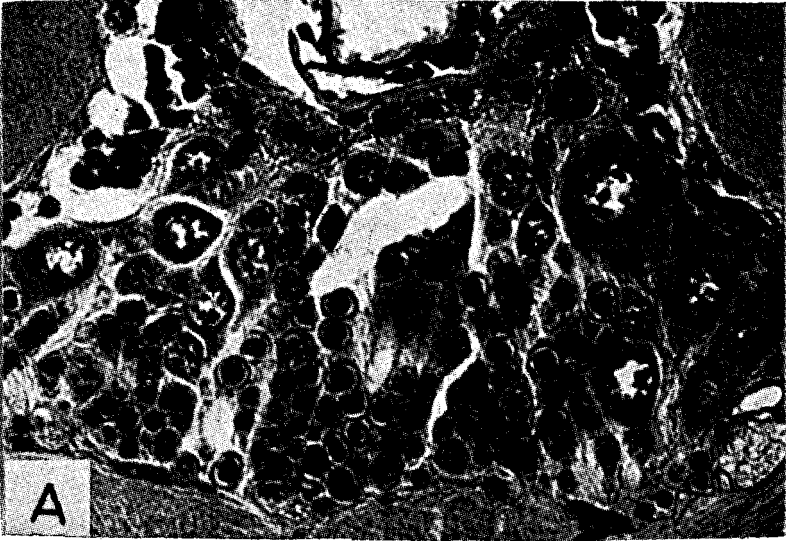
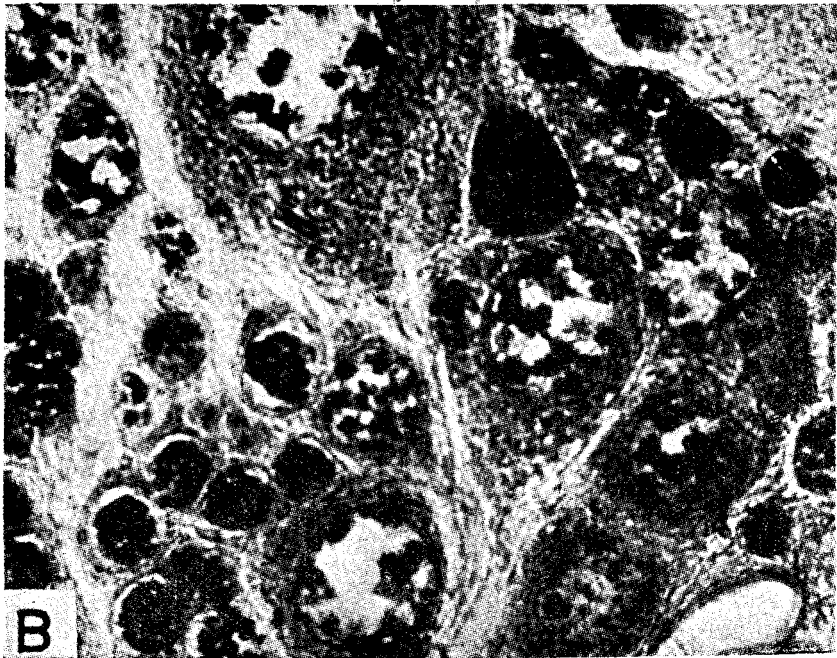


Figure 8. Cross-section through corpus allatum of fourth nymphal instar ♂ *Acrida*, killed 7 days after moulting. ns, normal secretory cell; u, undifferentiated cell; gs, giant secretory cell; ms, connective tissue cell.



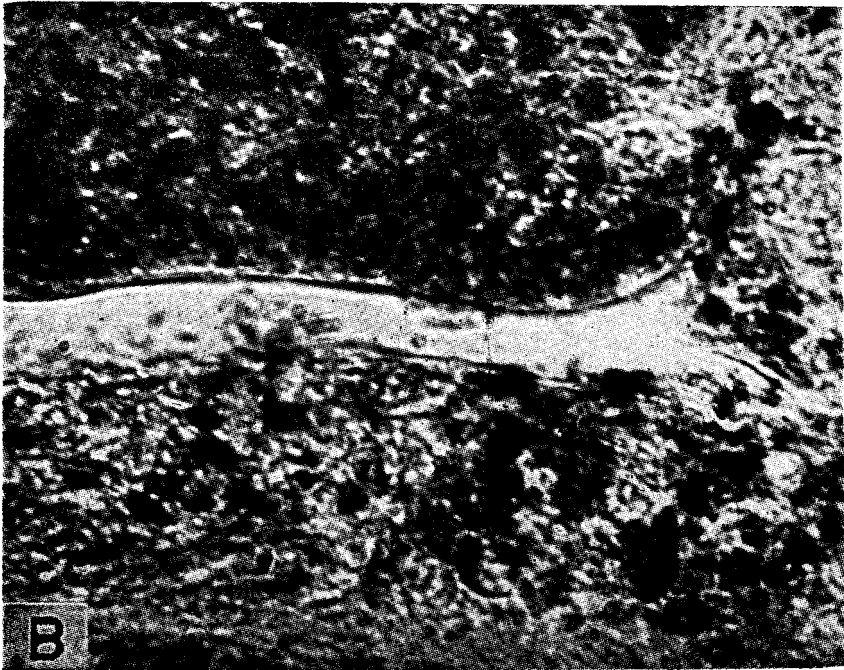
A — Pars intercerebralis of fifth nymphal instar ♂ *Locusta*, killed 3 days after moulting. Bouin, chrome hematoxylin - eosin. Photomicrograph x450.



B — Neurosecretory cells in the pars intercerebralis of fifth nymphal instar ♂ *Locusta*, killed 3 days after moulting. Bouin, chrome hematoxylin - eosin. Photomicrograph x1000.



A — Cross - section through corpora cardiaca and hypocerebral ganglion of fifth nymphal iastar ♂ *Locusta*, killed 2 days afreer moulting. Bouin. cchrome hematoxylia - eosin. Photomicrograph x100.



B — Cross - Section through corpora cardiaca of fifth nymphal iastar ♀ *Locusta*, killed 2 dazs after moulting. Neurosecretory material accumulated in the corpora cardiaca. Bouin, chrome hematoxylin - eosin. Photomicrograph x10000.



C — Section through cerebral ganglion of adult ♀ *Locusta*, showing neurosecretory material in the axons of the nervi corporis cardiaci 1. Bouin, chrome hematoxylin - eosin. Photomicrograph x450.

Fifth nymphal instar

The corpora allata attain their maximum size in the last nymphal instars of both sexes. At this stage the average dimensions of the corpora allata are as follows:

<i>Locusta</i> , Male:	204 μ long	—	130 μ wide
	Female:	247 > >	— 160 > >
<i>Acrida</i> , Male:	148 > >	—	93 > >
	Female:	170 > >	— 118 > >

In *Locusta* the duration of the fifth nymphal stage at 27°—29°C is about seven or eight days. Activity of the gland at this stage is generally similar to the activities of earlier stages. All cells are alike and no activity takes place during first three days after moulting. Mitosis begins in the third day and continues until the fifth days (Fig. 9). However, mitotic activity reaches its maximum at the fourth day. Both normal and polyploid mitoses are found.

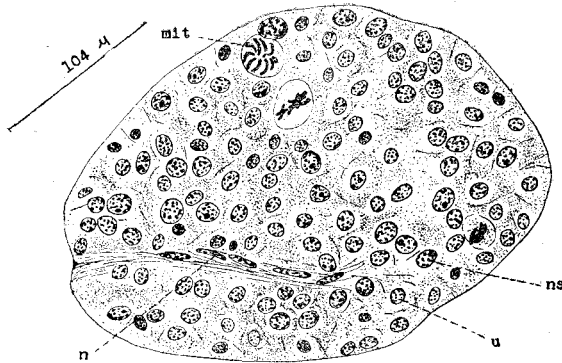


Figure 9. Cross-section through corpus allatum of fifth nymphal instar ♀ *Locusta*, killed 3 days after moulting. ns, normal secretory cell; u, undifferentiated cell; n, nerve cell; mit, mitosis in normal secretory cell.

The quantity of acidophil granules in the gland cells is increased on the fifth and sixth day, but it can never reach the quantity of fourth instar. At this stage the period of secretory activity of the gland is comparatively short. The gland cells contain some granules at the end of the stage, but the vacuoles are empty (Fig. 10) and the "lines of flow" are apparent.

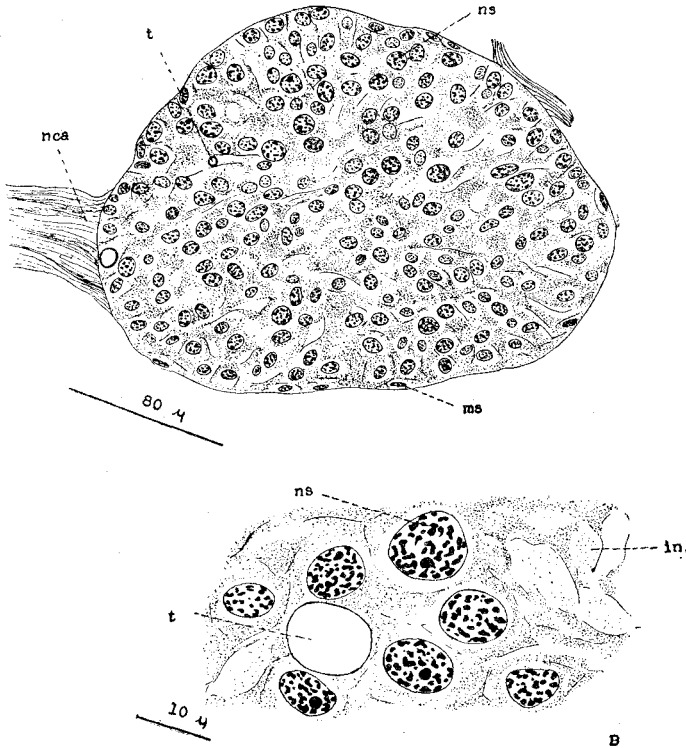


Figure 10. A-Cross-section through corpus allatum of fifth nymphal instar ♂ *Locusta*, killed 7 days after moulting. B-Enlarged cells. nca, nervus corporis allati; t, trachea; ns, normal secretory cell; ia, intercellular vacuole; ms, connective tissue cell; u, undifferentiated cell.

B-In adults

The average dimensions of corpora allata are as follows :

<i>Locusta</i> , Male :	250 μ long	—	160 μ wide
	Female :	305 μ long	— 224 μ wide
<i>Acrida</i> , Male :	194 μ long	—	140 μ wide
	Female :	232 μ long	— 162 μ wide

The gland is not active during the seven or eight days after the molt. The cells are similar and contracted. Inter-cellular vacuoles are wide and empty. A few acidophil granules are

seen in the gland cells (Fig. 11). The number of giant cells is greatest in the corpora allata of mature insect. A gland may

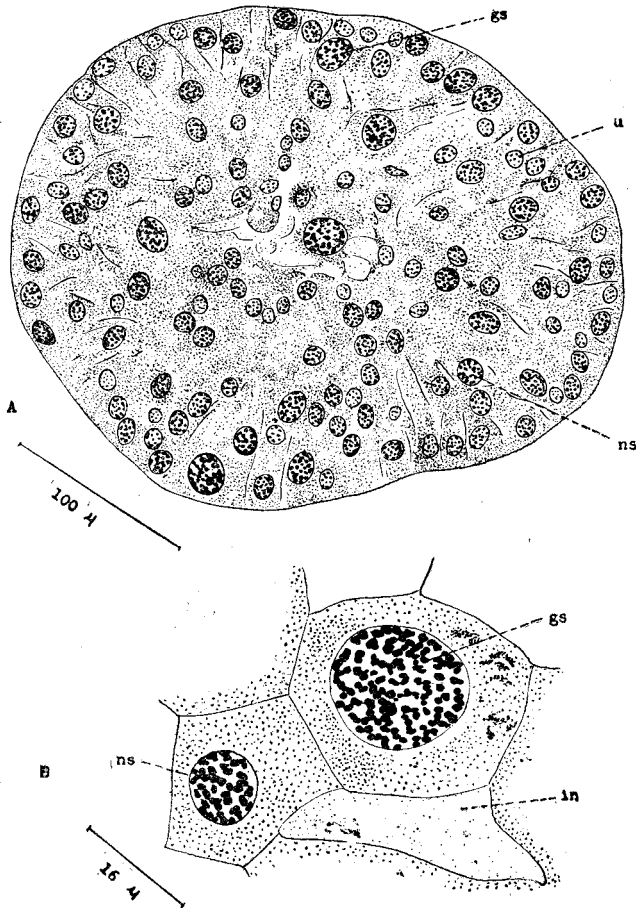


Figure 11. A-Cross-section through corpus allatum of adult ♀ *Locusta*, killed 3 days after moulting. ns, normal secretory cell; u, undifferentiated cell; gs, giant secretory cell; in, intercellular vacuole. B-Enlarged cells.

have up to eight polyploid cells, but the average number of giant cells is five. Although the corpus allatum continues to grow during every nymphal stage, there seems to be no mitotic activity in the glands of adult insects; consequently no growth takes place. This indicates that the development of the gland is completed in the fifth nymphal stage. It is concluded from the

fact that the number of giant cells in mature grasshoppers is more than in immature insects. Mitosis in these cells is still taking place. An increase in the amount of acidophil granules is observed in normal and giant secretory cells in the ninth and tenth days after moulting. This is the period when both sexes become mature. The cytoplasm and the vacuoles are filled with acidophil granules (Fig. 12).

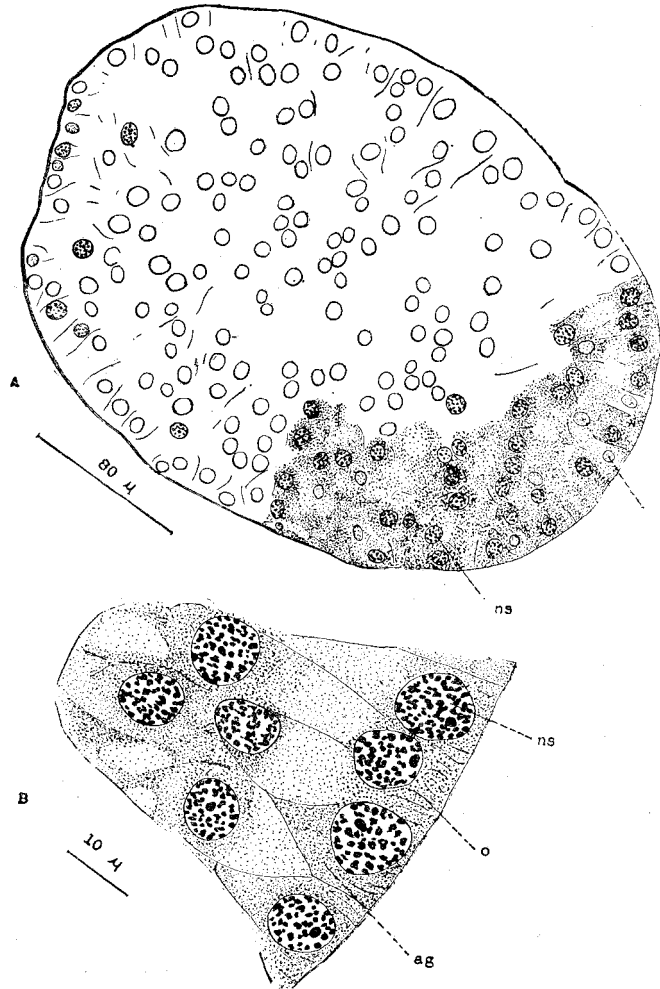


Figure 12. A-Cross-section through corpus allatum of mature ♀ *Locusta*, killed 12 days after moulting. B-Enlarged cells. ns, normal secretory cell; u, undifferentiated cell; o, "lines of flow"; ag, acidophil granules.

The duration of the activity of the gland at this stage is longer than at other stages. It is found that when the sexual activity of grasshoppers ends, the activity of the corpus allatum comes to an end as well. Secretory cells of old males and females are empty and contracted and empty vacuoles are produced among the cells (Fig. 13).

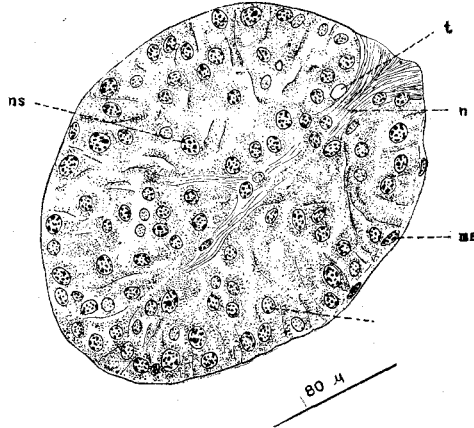


Figure 13. Cross-section through corpus allatum of sexually mature adult ♀ *Acrida*. ns, normal secretory cell; t, trachea; n, nervus corporis allati; ms, connective tissue cell; u, undifferentiated cell.

VI — Discussion

Histology of the gland: Casal and Guerrier (1946) make reference to only one type of cell, apart from the connective tissue cells in various Orthoptera species. The authors in question called the above connective tissue cells "Cellules parenchymateuses". They are secretory cells.

According to Joli (1945) the corpora allata of *Dytiscus* (Orthoptera) contain two types of cells. They are connective tissue cells and secretory cells.

The gland of the both species of Orthoptera which we investigated is composed of four types of cells. They are: a) connective tissue cell, b) undifferentiated cell, c) secretory cell and d) giant secretory cell. This result confirms the researches made on *Melanoplus* (Mendes 1948) and on other species of grasshoppers.

The intercellular vacuoles could not be seen in *Locusta* and *Acrida* as seen by Mendes in *Melanoplus* during the most active period of the gland, but these intercellular vacuoles have been observed at the time when the gland completed its activity, the cells being emptied and contracted. Wigglesworth (1934) could not see vacuoles in *Rhodnius* when the gland was active but saw them in their inactive period. This difference which is observed between *Melanoplus*, *Locusta* and *Acrida* may be due to the difficulty in differentiating intercellular vacuoles (which are filled with secretory granules in their active period) from cytoplasm.

In *Melanoplus*, *Locusta* and *Acrida*, at periods of greatest activity, lines of flow extending from center to periphery are seen among the periphery cells.

Giant secretory cells are found in every stage of the male and female nymphs. Reference was also made by Mendes to these cells which are formed as a result of polyploid mitosis. It is estimated that in *Acrida* and *Locusta* (according to number of nucleoli and size of nucleus) the polyploid cells are mostly tetraploid compared with the observation of octoploid cells in *Melanoplus* (according to number of chromosomes). The nuclei of giant cells are always regular and round shaped. The polymorph nuclei mentioned by Mendes have not been observed.

The sizes of the corpora allata in different nymphal instars show that its development is approximately proportionate to the development of the body. The sizes of adult corpora allata are three times bigger as compared with those in third nymphal instar. This rate is equal to the development rate observed in *Dixippus* (Pflugfelder 1936).

The activity of the gland: The activity of the corpus allatum has so far been investigated chiefly from the physiological point of view. The results obtained by Wigglesworth in *Rhodnius*, (1934, 1936, 1940, 1948), were confirmed for *Dixippus* (Pflugfelder, 1937), for *Melanoplus* (Pfeiffer, 1939) and for *Leucophea* (Sharrer, 1946). According to the above mentioned authors the corpora allata secrete a juvenile hormone. This hormone helps to preserve nymphal characters. Extirpation of this gland from a young nymph after one or two moults causes the insect to pass to the adult stage. As a result of this

function nymphs which moult less often than normal become dwarf adults. The transplantation of gland to an insect in the last nymphal instar helps to preserve nymphal characters longer. As a result of the increased number of moults giant insects come into being. The existence of a special hormone serving metamorphosis is only known in *Rhodnius* and not in other Hemimetabolone insects. It is agreed that the adult characters in the nymph are latent and not active.

a) **In nymphal instar** : The observation we carried out on the cross sections of the corpora allata definitely showed that the gland possesses a periodic activity in different nymphal instars. No activity can be seen in the gland in the first two or three days after moulting. After that mitosis divisions are seen and in the meantime the gland grows. Later on secretion starts. When the gland is empty the activity stops again. The secretory material is very little in the cross-sections of corpora allata taken prior to moulting. This demonstrated that almost all secretory material or juvenile hormone prior to the moulting process, was mixed with the blood. This periodical activity occurs in the same way in *Locusta* and *Acrida* species. These results confirm the histological observation made on *Melanoplus* (Mendes, 1948), *Dixippus* (Pflugfelder, 1937) and (Wigglesworth, 1934).

According to the histological researches made on *Locusta* and *Acrida* the period of greatest activity of the gland is the fifth and sixth days of the fourth nymphal instar. The authors working in physiological circles agree on the existence of a critical period in the life of the nymph. Experiments on extirpation show that if this operation is performed before the critical period, its effect can immediately be observed. If the extirpation is carried out after the critical period no effect could be seen. The extirpation of the gland from *Dixippus* during its last nymphal instar does not play any important part in the development of the insect (Pflugfelder, 1937-1938). The critical period in *Rhodnius* was observed in the fourth instar (Wigglesworth, 1937) in *Melanoplus* in the second half of the fifth stage (Mendes, 1948). Our observations show the existence of a critical period in *Locusta* and *Acrida* which coincides with the second half of its fourth nymphal stage.

Secretion is observed during the fifth instar (the last nymphal instar) but the activity of secretion in this stage starts late and does not last long. The quantity of acidophil granules accumulated in the gland is small. The extirpation of the corpora allata from *Dixippus*, *Melanoplus* and *Leucophea* in this stage does not effect metamorphosis. In the last nymphal instar it is considered that the gland stops secreting. We have observed, a very short, period of active secretion in the last nymphal stage of *Acrida* and *Locusta* on which we carried out investigations. This result confirms the observations made by Mendes (1948) in *Melanoplus*. This author has pointed out that the secretion of the gland in the last nymphal instar of the grasshopper in question was not adequate to prevent metamorphosis. This explanation may also be acceptable for *Acrida* and *Locusta*. On the other hand Bodenstein (1943) in *Drosophila* and Sharrer (1946) in *Leucophea* have determined that in their last nymphal instar tissues show less reaction against juvenile hormone than the other instars.

b) In adult: The corpora allata of the male and female of the two different species on which investigations were carried out did not show any activity during the first two weeks following the moulting process; that is, until they reach sexual maturity. The gland becomes active when the insect is mature. We have not observed any mitotic process in *Acrida* and *Locusta* before the secretion starts as observed in some of the mature Orthoptera species by Casal and Guerrier and by Mendes. The absence of mitotic activity shows that the gland does not increase further.

The beginning of secretion of the corpus allatum in the sexually mature insect shows that this gland then possesses other functions. The secretion continues until the end of sexual activity. Therefore the secretion in the mature insect is not a periodic activity. The corpus allatum especially in mature female, reaches a high level of secretory activity where it remains until cessation of sexual activity.

Physiological researches have been made to find out whether the corpus allatum in adults secretes a different hormone than in the uymph. It has been found in the reserches made on *Dixippus* (Pflugfelder, 1940) and *Melanoplus* (Pfeiffer, 1945)

that the transplantation of adult corpora allata to the last or other nymphal instars causes an over moulting and as a result of this a delay in metamorphosis. We therefore came to the conclusion that the secretion of the mature gland is the same as the secretion of the nymph.

According to the observations we made the acidophil granules constituting the secretion of nymphs and adults of both species are the same as each other. This result confirms the histological researches made (Mendes, 1948) on *Melanoplus* adults.

The origin of secretion: Cazal and Guerrier (1946) mention that the secretion which has been seen in the cell in the form of a drop or plate is probably formed by mitochondria. In the investigations we made on *Acrida* and *Locusta* this plate was not seen. The secretion was in the form of acidophil granules as found by Mendes in *Melanoplus*. These granules are not only found in regions near the mitochondria but in every part of cytoplasm. It would therefore be more reasonable to accept that the secretion is directly formed of the substances composing the structure of cytoplasm. As to probability of the secretion of more than one hormone by the corpora allata each of a different nature; it may be pointed out that there is no histological evidence for this.

VII — Summary

1 — The corpora allata and corpora cardiaca are investigated morphologically in the following species: *Bolivaria brachyptera* (Pall.), *Saga cappadocica* (Wern), *Bradyporus dilatatus* (St.), *Acrida bicolor* (Thund.), *Locusta migratoria* (L.) Ph. Solitaria, *Glyphotmethis escherichi* (Kr.).

2 — Although there are some morphological differences between the retro-cerebral glands in different species, those of the species belonging to the same family are rather similar.

2 — The corpora allata are studied histologically in *Locusta* and *Acrida*. The cellular elements of this gland in the nymphs and adults are as follows:

a) Connective tissue cells: These are found on the periphery of the gland and have an elongated nucleus.

b) Undifferentiated cells: These are few in number and possess small quantities of chromatin. They show a strong mitotic activity at the beginning of each nymphal stage.

c) Normal secretory cells: These form a large part of the gland and resemble the undifferentiated cells when they are not active. Their nuclei have an abundant quantity of chromatin. When they begin to secrete they become more voluminous and their cytoplasm is filled with acidophil granules. In this stage inter and intra-cellular vacuoles are formed.

d) Giant secretory cells: Three to eight polyploid cells are found in each gland. Their activity is similar to that of normal secretory cells.

e) The cells of the nerve to the corpus allatum have a large quantity of chromatin.

4 — The cyclic activity of the secretory cells is as follows: The cells are not active in the nymphs during the days immediately after moulting. Mitotic activity is then observed in the gland. Finally the secretory cells are filled with acidophil granules and vacuoles are formed. The release of secretion occurs in the second half of the stage. The cells shrink at the end of the instar. Very few granules are seen in this stage. The critical period lays between the sixth and seventh days of the fourth-stage in the species studied. Secretory activity during the last (fifth) nymphal stage is shorter and less pronounced.

5 — No activity is observed after the emergence of the adult until the beginning of sexual maturity. Mitotic divisions are always absent in this instar. Secretion begins just before the ripening of the eggs in the females and reaches its highest level. This level is maintained until the end of sexual maturity. Similar activity is observed in adult males.

6 — These results confirm the previous physiological investigations carried out in different groups of insects and the histological studies made in *Melanoplus*.

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