Two Kinds of Secretions in Corpora Cardiaca of *Locusta migratoria* (L) Ph. Solitaria

(Orthoptera : Acrididae)

by

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Özet : 1 — *Locusta Migratoria* (L) Ph. Solitaria'da corpora cardiaca ve beynin pars intercerebralis bölgesindeki nörosekresyon hücreleri histolojik olarak incelenmiştir.

2 — Her iki cinsin larva ve erginlerinde corpora cardiaca dört tip hücre ihtiva eder :

a — Mezenşim hücreleri, b — Farklılaşmamış hücreler, c — Salgı hücreleri, d — Sinir hücreleri.

a — Mezenşim hücreleri : Bezin etrafını çeviren uzun çekirdekli örtü hücreleridir.

b — Farklılaşmamış hücreler . Mitozla çoğalırlar, Kromatini bol gayri muntazam çekirdeklidirler.

c — Salgı hücreleri : Bezin esasını teşkil ederler. Çekirdekleri yuvarlak ve açık renk olan bu hücrelerin sitoplazmaları daima salgı granülleri ile doludur. Larva safhalarının başında mitozla çoğalırlar.

d — Corpora cardiaca'yı baştan sona kat'eden bazal sinirden bezin her tarafına sinir fibrilleri uzanır. Fibriller arasında sinir hücrelerinin çekirdeklerine rastlanır.

3 — Corpora cardiaca'da devri bir faaliyet müşahede edilmemiştir. Dişi ve erkek fertlerin gerek larva, gerek erginlerinde salgı hücrelerinde daima bol miktarda ifraz granülüne rastlanmıştır.

4 — Beynin pars intercerebralis bölgesindeki salgı hücreleri tarafından devri bir faaliyetle nöroksekresyon madde meydana getirilir. Sitoplazmada granül halinde görülen bu salgı Gomori'nin chrome-alum-hematoxylin-phloxin metodu ile koyu maviye boyanır.

5 — Pars intercerebralis'in nörosekresyon hücreleri tarafından meydana getirilen bu salgı corpus cardiacum sinirlerinin aksonu'u boyunca ilerliyerek corpora cardiaca'da depo edilir. Bu salgı corpora cardiaca hücreleri arasında iri plak ve damlalar halindedir. Nörosekresyon salgı hiçbir zaman corpora allata'ya geçmez

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I — Introduction

The neurosecretory material in the corpora cardiaca of different species of insects have been observed by many scientists, but observations on the structure and activity of the corpora cardiaca are yet very few.

The structure of the corpora cardiaca was investigated by Cazal and Guerrier (1946) in Orthoptera, Joly (1945) in Dytiscidae and Nayar (1954) in *Locusta migratoria*. The neurosecretory cells of the pars intercerebralis and their activity were investigated histologically by Hanström (1953) in Apterygota, Stutinsky (1952) in some species of insects, Scharrer (1952) in *Leucophaea maderae*, Bounhiol, Gabe and Arvy (1953) in *Bombyx mory*, Gabe and Arvy (1954) in some Plecoptera, Gabe (1953) in Thysanour and (1955) in Arachnida, De Lerma (1956) in *Hydrous piceus* L. (Coleoptera). They found a secretory material stainable with chrome-alum-hematoxylin-phloxin of *Gomori* (1941) in pars intercerebralis of the brain. The same authors concluded that: This neurosecretory material which is secreted by neurosecretory cells of the pars intercerebralis passes to the corpora cardiaca through the axon of the nervis corporis cardiaci I and it is accumulated in the corpora cardiaca. This neurosecretory material is carried into the corpora allata in *Bombyx mori* (Bounhiol, Gabe, Arvy, 1953) and in some Plecoptera (Gabe, Arvy, 1954).

Our histological investigations were made on the secretory cells of the corpora cardiaca and pars intercerebralis in *Locusta migratoria* (L) Ph. Solitaria. The structure and activity of the corpora cardiaca and the neurosecretory cells of the pars intercerebralis in nymphal stages and adults were examined by two different staining methods. The results of these investigations were compared with results obtained by other investigators from different species of insects.

II — Material and Method

The observations were made on *Locusta migratoria* (L) Ph. *Solitaria* which were cultured in our laboratory. The method was similar to that of earlier experiments (Özbaş 1957). The heads of adults and nymphs of different stages were fixed in

Bouin and serial cross sections 6 - 7 microns thick were taken. The corpora cardiaca were stained with hematoxylin - eosin and neurosecretory material by chrome - alum - hematoxylin - phloxin of G o m o r i (1941) (in this method eosin was used instead of floxin, because it was impossible to obtain floxin).

III — Observations

A — The structure of the corpora cardiaca

The location and morphological structure of the corpora cardiaca in the head of *Locusta* were discussed in detail in an earlier paper (Ö z b a ş 1957). Now only the histology of the corpora cardiaca will be explained. The structure of the gland is complex, but can be classified as follows:

a) Connective tissue cells, b) Undifferentiated cells, c) Secretory cells, d) Nerve cells, e) Tracheae.

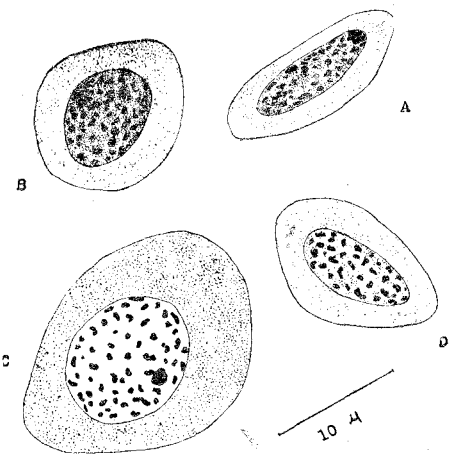

Figure 1. Types of cells in corpora cardiaca.

A — Connective tissue cell (fifth instar ♀, killed 8 days after moulting).

B — Undifferentiated cell (fourth instar ♂, killed 5 days after moulting).

C — Secretory cell (fourth instar ♂, killed 5 days after moulting).

D — Nerve cell (adult ♀, killed 8 days after moulting).

a — Connective tissue cells (Fig. 1, A)

These cells are located on the periphery of the gland, as a single layer of epithelium tissue. The thin layer of tissue which

connects the corpora cardiaca with one another dorsally is composed of these long, flat cells. The major diameter of the nucleus is 10 microns. The nucleus has a large amount of chromatin giving it a dark coloured appearance. This layer is in contact with the surface of the aorta along the inner surface of the corpora cardiaca.

b — Undifferentiated cells (Fig. 1, B)

These cells are present in every stage in the development of the corpora cardiaca. Their cytoplasm is light in colour. On the other hand their nuclei are very dark and they are stained deeply with hematoxylin. These dark nuclei are elipsoidal or some of them are in irregular shape and their diametres are about 8 microns. Although located everywhere in the gland, they are found mostly on its periphery.

c — Secretory cells (Fig. 1, C)

The major part of the gland is made of these cells, being present in the nymphs and adults in large numbers. Their size is II microns and have round nucleus. They are easily distinguished from other cells by means of the small chromatin granules in their nuclei. The nuclei are lighter, in comparison with other cells. Their cytoplasm is acidophil and stained rose in eosin and always has acidophil granules.

Strong mitotic activity is seen at the beginning of every nymphal stage. The corpus cardiacum does not have any giant secretory cells. Mitotic activity was not seen in any adult *Locusta* which was under observations.

d — Nerve cells (Fig. 1, D)

A pair of nerves coming from the protocerebrum section of the cerebral ganglion enter the corpora cardiaca anteriorly and pass through these glands. They are called 'basal nerves'. Each of them branches into two nerves, one to cerebral ganglion and the other to the corpus allatum. Moreover, many small nerves which originate in the basal nerves reach the periphery of the gland.

The nuclei of the nerve cells are long and rich in chromatin, the major diameter of the nucleus is about 10 microns. They are very similar to connective tissue cells but are identified very easily, because of their different location in the gland.

e — Tracheae

Tracheae are found in the vicinity of the nerves in the corpora cardiaca.

B — Activity of the corpora cardiaca in the nymphal instars and adults.

a — In nymphal instars

It was found that the activity which takes place in the corpora cardiaca is the same in different nymphal instars. The corpora cardiaca were not active in the nymphs immediately after moulting.

The quantity of acidophil granules in the cytoplasm of the secretory cells is rather small. The secretory and undifferentiated cells are active on the second and third day after moulting. At this time both the nuclei and the cytoplysm of the cells begin to grow, in preparation for the mitotic activity.

Mitotic activity is at a maximum on the third and fourth day after moulting (Fig. 2).

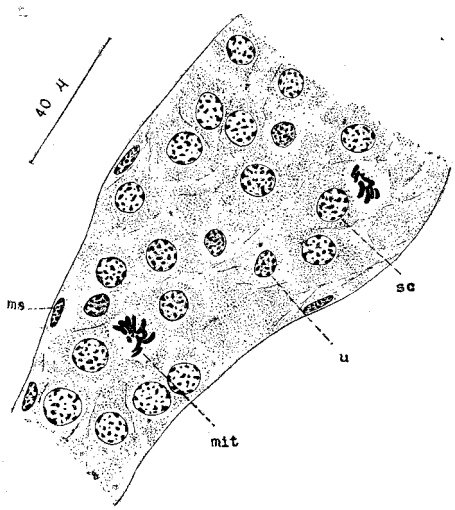

Figure 2. Cross-section through corpus cardiacum of fourth nymphal instar ♀ *Locusta*, killed 3 days after moulting. ms, connective tissue cell; *u*, undifferentiated cell; mit, mitosis. Bouin, hematoxylin-eosin.

The quantity of acidophil granules in the secretory cells begins to increase after this mitotic activity. An abundant quantity of granules is seen until the end of the instar. This is

different from corpora allata (Fig. 3). Although the acidophil granules are discharged from the cells, their quantity does not decrease because the secretion is continous.

b — In adults

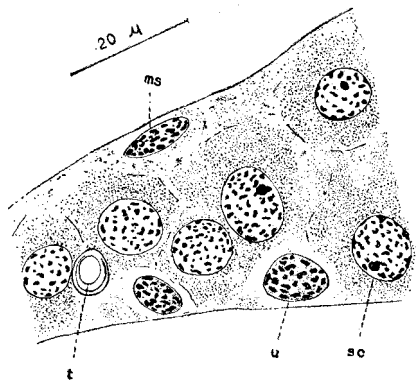

Figure 3. Cross-section through corpora cardiaca of fifth larval instar ♀ *Locusta*, killed 6 days after moulting. u, undifferentiated cell; sc, secretory cell; t, trachea. Bouin hematoxylin-eosin.

The corpora cardiaca are not active in adults, immediately after moulting. Mitotic activity is not present, because the gland reaches its normal size in the last nymphal instar. The quantity of granules begins to increase in the third and fourth days of the instar. Intracellular and intercellular vacuoles are present.

Many granules were always found in the secretory cells of male and female *Locusta*. The quantity of granules in older individuals is somewhat decreased.

C — The neurosecretory cells of the pars intercerebralis and their activity.

The neurosecretory cells of the pars intercerebralis in the protocerebrum are located on both sides of the median line. They are rather large cells resembling pears, the nucleus which is round contains large chromatin granules and is located exactly at the center of cell. When cytoplasm was stained by the chrome-alum-hematoxylin method, round dark blue neurosecretory granules appeared. It was impossible to determine the cell boun-

dary, especially ventrally where neurosecretory granules flow downward (Fig. 4, 5, Plate I A, B) Intracellular and intercellular vacuoles are present. The activity of the cells is periodical.

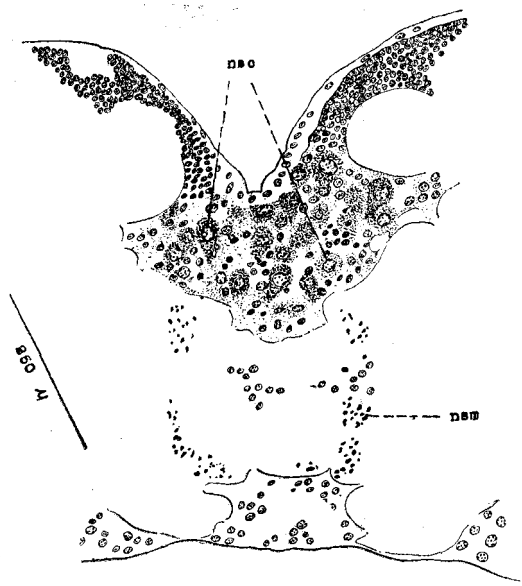

Figure 4. Section through cerebral ganglion of adult ♀ *Locusta*. nsc, neurosecretory cells in the pars intercerebralis; nsm, neurosecretory material in the axon of the nervi corporis cardiaci I. Bouin, chrome hematoxylin-eosin.

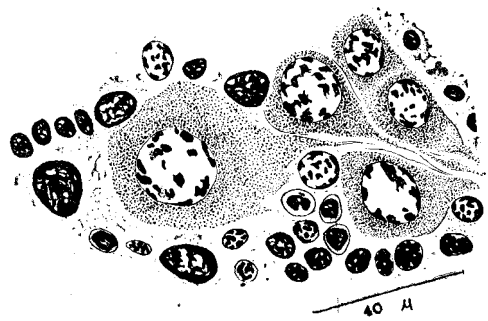

Figure 5. Neurosecretory cells in the pars intercerebralis. Fifth nymphal instar ♂ *Locusta*, killed 3 days after moulting. Bouin, chrome hematoxylin-eosin.

The neurosecretory material is always ample in the cells up to three days after moulting, as seen in the sections of larval

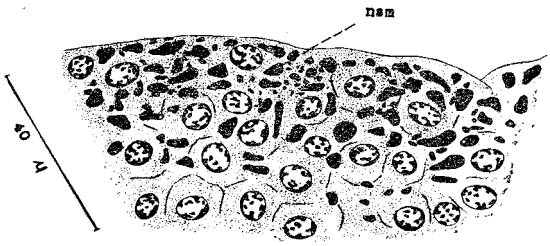

Figure 6. Neurosecretory material in the corpus cardiacum. Fitfh nymphal instar ♀ *Locusta*, killed I day after moulting. nsm, neurosecretory material. Bouin, chrome hematoxylin-eosin.

stages and adults. Discharge of the neurosecretory material takes place after this stage. At the end of the stage the quantity of neurosecretory granules became less and it is observed that the number of empty intracellular and intercellular vacuoles is increased.

The neurosecretory material in the corpora cardiaca :

The neurosecrotory product migrates along the axon of the nervi corporis cardiaci I and accumulates between the cells of the corpora cardiaca.

Neurosecretory material, stained, dark blue with chrome-alum hematoxylin, was observed in the form of discs and droplets in the axon of the nervis corporis cardiaci I (Fig. 4, Plate I : C).

The same neurosecretory material is also present between the cells of the corpora cardiaca of *Locusta* (Fig. 6, Plate II: B). This neurosecretory product is generally found in abundance in the corpora cardiaca on the side near the

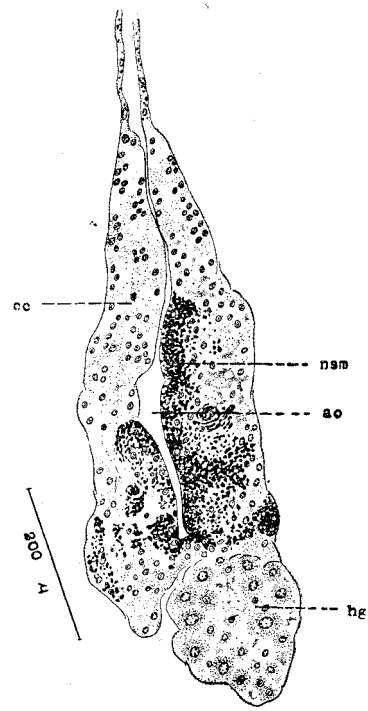

Figure 7. Cross-section through corpora cardiaca and hypocerebral ganglion of fifth nymphal instar ♂ *Locusta*, killed 2 days after moulting. cc, corpora cardiaca ; nsm, neurosecretory material ; ao, aorta ; hg, hypocerebral ganglion. Bouin, chrome hematoxylin-eosin.

neighbouring one (Fig. 7, Plate : II A). This neurosecretory material is deposited in the corpora cardiaca.

Chrome-hematoxlin did not stain the secretory material of the corpora cardiaca. The amount of secretory material increases proportionally as the corpora cardiaca grow. However this increase is not periodic. This material never passes to the corpora allata. It was observed that in the cross sections which were taken from the corpora allata of adult and nymphal *Locusta*, the neurosecretory material was not present.

IV. Discussion

A — Corpora cardiaca

Histology of the gland : There have been investigations of the structure of the corpora cardiaca. It was investigated by Cazal and Guerrier (1946) in three species of Orthoptera (*Ephippiger*, *Metrioptera* and *Orthacanthacris*). They found three types of cells in the gland. They are : Chromophil, chromophobe, and interstitielle cells probably correspond to the gland cells which were observed in *Locusta* by the author. Although no dendroidal forms of cytoplasm were observed, as indicated by the above authors, chromophobe cells probably correspond to the undifferentiated cells as found by the author. In these cells the quantity of chromatin is less than in the secretory cells, and they are smaller, their nuclei being irregular in shape. Interstitielle and nerve cells were found to be similar to those observed by Cazal and Guerrier. The secretory materials observed by these authors were in the form of plates and droplets. On the other hand the secretory materials of the corresponding cells in *Locusta* were found to be in the form of granules. The secretory cells of *Locusta* are equivalent to the fundamental cells of the corpora cardiaca (cellules fondamentales des corpora cardiaca) which were investigated in Dytiscidae by P. Joly (1945). The special cells which contain phloxinophyl granules and were observed by Bounhiol, Gabe and Arvy (1953, b) in *Bombyx*, Gabe and Arvy (1954) in Plecoptera, Gabe (1953) in Machilidae, Hanström (1953) in Apterygota, De Lerma (1956) in Coleoptera (which were not stained with chrome-hematoxylin) correspond to the secretory cells of *Locusta*.

Nayar (1954) described the corpora cardiaca of *Locusta migratoria* as a cyncytium containing nuclei of two sizes. Because a phase-contrast microscope was not used for our experiments the spheroid bodies in cytoplasm which were observed by the above author could not be seen.

Activity of the gland: The activity of the secretion is not periodical in the corpora cardiaca. The quantity of granules decreased slightly after moulting in nymphs and adults, but the secretion is always found in the gland.

Mitosis takes place during the first and second days of each nymphal stage but this is not seen in adults.

The role of the corpora cardiacaca is not known exactly. Few physiological experiments have been conducted on these glands. This may be because of the difficulty in extirpation and isolating the gland.

Joly (1945) and E. Thomsen (1948 b) wrote that this gland has a role in the development of egg-chambers. When extract of the gland is injected into Crustacea, their chromatophores contract (Hanström, 1940; M. Thomsen, 1943). The extract of Orthoptera is most effective among insects.

M. L. Cameron (1953) showed that the extract of corpora cardiaca has an influence on the pulsation of the heart and peristalsis of intestines in *Periplaneta americana* and *Locusta migratoria*. Cameron had repeated his experiments five and seventeen days after he had cut the nerves connecting the corpora cardiaca with the brain and had not seen any difference in the effect of the extract of the gland, According to Cameron it is probable that the active material is produced in the gland. This result confirms that the secretory material is present in the corpora cardiaca of the same species (*Locusta*) which we examined histologically.

Whatever the function or functions of the corpora cardiaca histological studies showed that this gland has a continious but not a periodical secretory activity in *Locusta*.

B — Pars intercerebralis

The neurosecretory cells of the pars intercerebralis described by M. Dupon-Raabe (1953, 1956) in Phasmidae, by Stutinsky (1952) in some species of insects and by

Sharrer (1952) in *Leucophaea maderae* were similar to those observed by the author in *Locusta migratoria* (L) Ph. Solitaria.

The dark - blue staining secretory material of the neurosecretory cells with chrome - hematoxylin method was observed by the above scientist and by Hanström (1953) in Apterygota *(Lepisma saccharina, Petrobius maritimus)*, Bounhiol, Gabe and Arvy (1953) in *Bombyx mori*, Arvy and Gabe (1954) in some Plecoptera, Gabe (1955) in Arachnida, De Lerma (1956) in Coleoptera and by the author in *Locusta*.

Activity of the cells : The activity of the neurosecretory cells is periodic in *Locusta* as in *Bombyx mori* (Bounhiol), Gabe and Arvy 1953 b). The secretion is abundant in the first days after moulting. A decrease in the amount of the secretory material is seen in the second half of the stage. This material, as observed by other authors, passes to the corpora cardiaca through N. C. C. I and it is accumulated there among the cells (Scharrer, 1952 ; Bounhiol, Gabe and Arvy, 1953 : Arvy and Gabe, 1954 ; De Lerma, 1956 ; M. Dupont-Raabe, 1956). The corpora cardiaca is a depot organ for neurosecretory material. A decrease of this material was not observed in the corpora cardiaca of *Locusta* ; on the other hand, the quantity of the material increased as the gland grew.

In *Locusta* this neurosecretory material did not pass to the corpora allata.

No effects of the neurosecretory product accumulated in corpora cardiaca on the insect have been found as yet.

Summary

1 — The secretory cells of the corpora cardiaca and the pars intercerebralis of the brain in *Locusta migratoria* (L) Ph. Solitaria were examined histologically.

2 — Corpora cardiaca : Four types of cellular elements are distinguished in *Locusta*.

a) Connective tissue cells : They are similar to those of the corpora allata. They are found on the periphery of the gland and have an elongated nucleus.

b) Undifferentiated cells: They divide mitotically. Their nucleus is of an irregular shape and possesses large quantities of chromatin.

c) Secretory cells: They form a large part of the gland. Their cytoplasm is always filled with granules. They divide mitotically at the beginning of each nymphal stage.

d) Nerve cells: The nuclei of the cells are long and rich in chromatin.

3 — A cyclic secretory activity is not observed in the corpora cardiaca. The cells are always filled with acidophil granules both in the nymphs and in the adults of both sexes.

4 — The neurosecretory material is produced periodically by the secretory cells of the pars intercerebralis of the brain. This material is stained dark - blue with chrome - hematoxylin of Gomori.

5 — The neurosecretory product migrates along the axon of the nervi corporis cardiaci I and accumulates between the cells of the corpora cardiaca. This secretory material never passes to the corpora allata.

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