The Neurosecretory Cells and Neurosecretions in the Developmental Stages of the Cotton Leafworm - Spodoptera Littoralis (B)

by

Mahmoud A. Banhawy

Department of Zoology, Faculty of Science, Qatar University, Doha, Qatar.
Faculty of Science, Ain Shams University, Cairo, Egypt.

and

Ibrahim M. Anwar

Department of Zoology, Assiut University, Assiut, Egypt

الخلايا العصيبة الافرازية والافرازات العصبية في المراحل التطويرية لدودة ورق القطن – سبودوبتيرا ليتوراليس

تأليف

الاستاذ الدكتور محمود البنهاوي و الاستاذ الدكتور ابراهيم انور

كلية العلوم – جامعة قطر

تم في هذه الدراسة تحديد وجود ثلاثة انواع من الخلايا الافرازية العصبية (أ ، ب ، ج) وذلك في المراكز العصبية للجهاز العصبي فيما عدا العقدة العصبية الجبهية للمراحل التطورية المختلفة لدودة ورق القطن .

وقد أمكن تتبع مظاهر النشاط لكل نوع من هذه الخلايا في جميع أجزاء الجهاز العصبي خلال دورة حياة الحشرة بدءاً بالاطوار اليرقية الأولى وانتهاءاً بالطور البالغ ثم المسن بما في ذلك طور العذراء الساكن .

وتبين من خلال الدراسات الهستوكيميائية على محتويات الخلايا الافرازية المختلفة في جميع مراحل التطور ان الافراز العصبي يحتوي على :

أ – مواد كربوهيدراتية تشتمل على الجليكوجين والمخاطيات الحمضية واستيرات الكبريتات عديدة التسكر .

ب – مواد بروتينية وتشتمل على الهستونات والتيروسين والتربيتوفين والأحماض الأمينية الكبريتية .

ج – حمض الاسكوربيك .

كذلك تم تتبع مراحل انتقال الافراز العصبي في اماكن تخليقه بالخلايا الافرازية وذلك خلال الألياف العصبية لهذه الخلايا ، ثم تجميعه داخل بعض التجاويف الموجودة خارج الخلايا في النسيج العصبي ثم انتقال هذا الافراز بواسطة الالياف العصبية للروابط الطولية للعقد العصبية وللأعصاب الجانبية الى اجزاء الجسم المختلفة .

Introduction

In 1955 Nayar described two types of neurosecretory cells in *Iphita limbata* (A- and B-types) [1]. Later, Johannson [2] classified these cells into four categories: A, B, C and D, which he regarded as motor neurones of neurosecretory function. Furthermore, Delphin [3] recognized in the ventral ganglia of *Schistocerca gregaria* four classes of neurosecretory cells (A, B, C and D), and subdivided A and B into A_1, A_2, A_3 and B_1, B_2, respectively. This was not accepted by the present writers [4], who considered that the different activity phases of their A-cells represent those identified by Delphin [3] as C- and D- types, and that the A_1, A_2, and A_3 cells of Delphin correspond to the activity phases of their B-cells, and that the phases of activity of their C-cells represent the B_1 and B_2 types of Delphin.

Banhawy and Anwar [4] reported that the neurosecretory cells and neurosecretory material in adult *Gryllotalpa gryllotalpa L.* are particularly rich in histones, SS and SH proteins, acid mucopolysaccharides and ascorbic acid.

The present work was planned to find out if there is any variation in the neurosecretory activity in the different developmental stages of *Spodoptera littoralis (B).*

Material and Techniques

Six successive developmental stages of *Spodoptera* bred under laboratory conditions were used in the present study. The dissected parts of the nervous system were fixed in Susa-picric fluid [3, 3, 6]. Bouin's and Carnoy's fluids were also employed.

For the identification of the neurosecretory cells, staining was carried out in Heidenhain's azan stain [7] and Ewen's paraldehyde fuchsin [8]. The method recommended by Delphin [6] involving the use of phloxine alcian blue was also applied.

The periodic acid Schiff (PAS) was employed for the demonstration of carbohydrates [9]. Glycogen was examined in sections treated with Best carmine. The method of Lison [10] was used to identify mucopolysaccharides; the acid components were shown by Hale's technique [11]. Polysaccharide sulphate esters were displayed following the procedure of Kiyoshi [12].

General proteins were demonstrated in material treated with the mercury-bromophenol blue technique as recommended by Mazia, Brewer and Alfert [see 13]. The method of Schneider [14] was used to demonstrate histone contents. Tyrosine was revealed by Bensley and Gersh's modification [15] of Millon's reaction, and tryptophane was detected in paraffin sections post oxidation in 5% chromic acid followed by staining in 1% eosin and then in 1% light green. Cystine (SS-protein) was detected in performic acid Schiff treated sections [13], whereas cysteine (SH-protein) was examined after the prussian blue method [16].

Lipoprotein and lipofuscin materials were shown according to Ziehl Neelsen carbol fuchsin method [16].

For the detection of lipoidal inclusions the technique of Gatenby and Moussa [17] was employed. Lecithin was examined post-treatment with Ehrlich haematoxylin followed by differentiation in borax-ferricyanide solution [16]. Schultz-Smith method was applied for cholesterol.

The pyronin-methyl green technique of Kurnick [18] and Feulgen reaction were em-

ployed for revealing nucleic acids. Ascorbic acid was detected with the methods of Bourne [19] and Barnett and Bourne [20].

Sites of acid and alkaline phosphatase activities were visualized in materials subjected to the reactions of Gomori [21, 22].

Results

Neurosecretory Cells

These cells first appear in the third larval ages of the insect. They arise from the undifferentiated cells of the early larval stages due to certain changes including the following: increase in the cell size, widening of the cytoplasm, appearance of cytoplasmic granules, and prominent nuclei with bright nucleoli.

The neurosecretory cells were observed in the different parts of the nervous system except the frontal ganglion. These cells are classified according to their sizes and staining abilities into three main types (A-, B- and C- types), as represented on Plate 1, Figs. 1, 2, 3 and 4. With Heidenhain's azan stain the A-cells stain violet, the B-cells take a reddish colour and the C-cells become mauve.

1. Type A: Represents the largest type in which the diameter of the cell varies from 6.2 to 13μ. In the median larval stages the cells have a mean cellular and nuclear diameter of 9.2μ and 4.4μ respectively. The nuclei are round, and each contains a few chromatin particles and a pair of distinct bright nucleoli of about 1.3μ in diameter. The average width of perikaryon is 4.8μ (Table 1). The ground cytoplasm is loaded with fine granules which stain violet with Heidenhain's azan stain and blue with Delphin's stain (Fig. 1).

Table 1
Measurements of the Neurosecretory Cells in the Successive Developmental Stages of Spodoptera littoralis

		Diameter (μ)						
Cell-type	*Stage*	*EL*	*ML*	*PP*	*P*	*Am*	*Om*	*Sm*
A	Cell	3.0	9.2	8.8	13.0	7.0	6.5	6.4
	Nucleus	2.0	4.4	4.0	6.0	4.5	5.0	5.5
	Nucleolus	0.4	1.3	1.3	1.5	0.7	0.4	-
	Perikaryon	1.0	4.8	4.8	7.0	2.5	1.5	0.9
B	Cell	3.0	6.5	6.0	5.0	6.5	5.0	5.0
	Nucleus	2.0	3.4	3.0	2.7	4.0	3.8	4.4
	Nucleolus	0.4	1.0	1.0	0.5	1.2	0.4	-
	Perikaryon	1.0	3.1	3.0	2.3	2.5	1.2	0.6
C	Cell	3.0	4.0	4.5	3.2	4.8	3.8	2.3
	Nucleus	2.0	2.6	2.4	2.0	3.2	2.8	2.0
	Nucleolus	0.4	1.0	0.8	0.4	0.7	0.4	-
	Perikaryon	1.0	1.4	2.1	1.2	1.6	1.0	0.3

EL, early larvae; ML, median larvae; PP, prepupae; P, pupae; Am, adult moths; Om, old moths; Sm, senile moths.

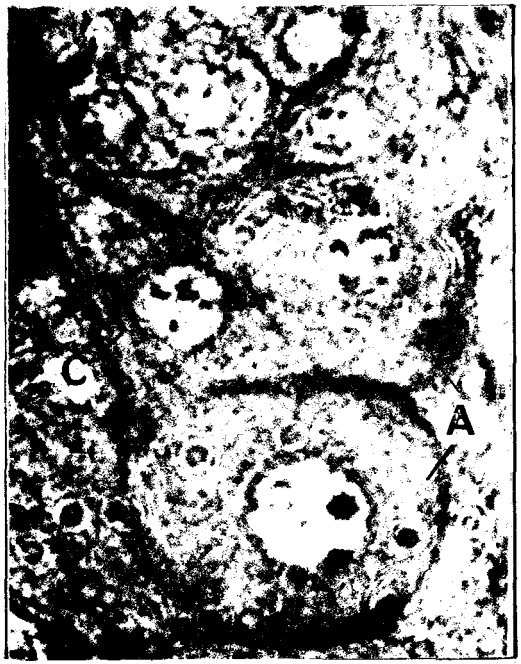

Fig. 1

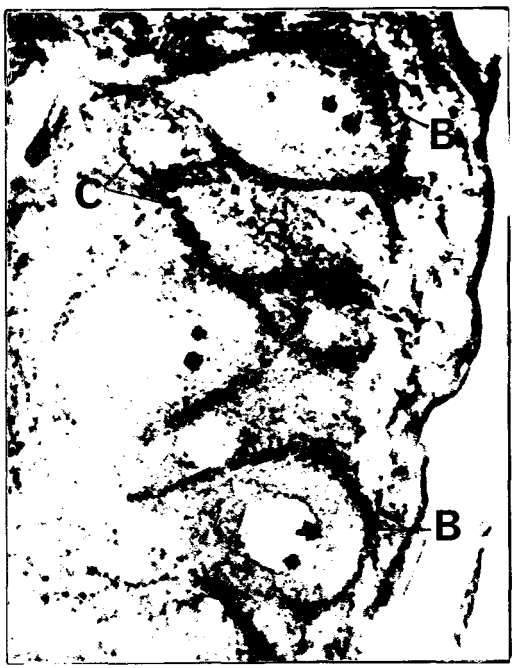

Fig. 2

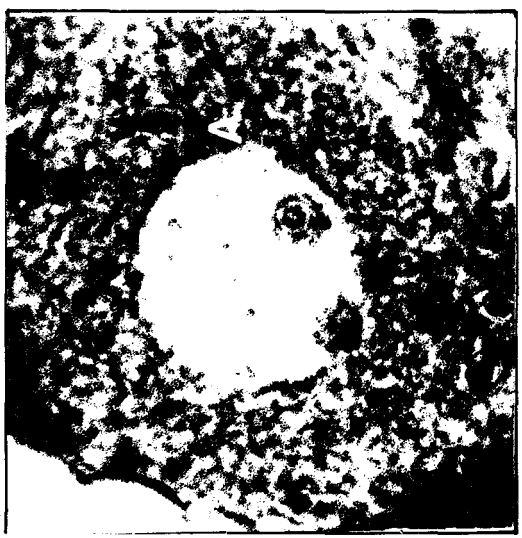

Fig. 3

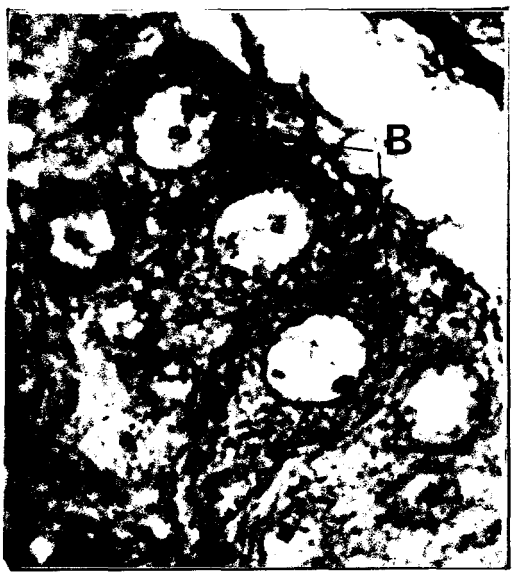

Fig. 4

In the prepupal stages, the A-type cells are somewhat reduced in size (Table 1), but are greatly enlarged during the pupal stages. The average diameters of the cell and nucleus are 13μ and 6μ respectively. Each nucleus contains a pair of prominent nucleoli with a mean diameter of 1.5μ. At these stages the cells show an active phase of neurosecretion (Fig. 3).

In moth stages the cells become smaller in size and show a moderate activity of synthesizing neurosecretory material, except in the forebrain where they occasionally show a strong activity. The average diameters of the cells, nuclei and nucleoli are 7.0, 4.5 and 0.7μ respectively.

In ageing insects, the size of the cells is gradually decreased and the size of the nuclei may become slightly larger; this results in a markedly narrow perikaryon ranging between 1.0μ and 1.5μ. The activity of the cells becomes remarkably weak.

2. Type B: The cells are slightly smaller than those of type A. In the median larval stages the mean cellular, nuclear and nucleolar diameters are 6.5μ, 3.4μ and 1.0μ respectively; and the width of the perikaryon is about 3.1μ. The cells contain numerous granules in the cytoplasm (Fig. 2).

During the prepupal and pupal stages the cells become gradually reduced in size and the content of the neurosecretory material becomes less. The average diameters of the cells, nuclei and nucleoli in the pupal stages are 5.0μ, 2.7μ and 0.5μ respectively (Table 1).

In moth stages the cells become larger in size and highly loaded with deeply stained cytoplasmic granules; this indicates that the cells are at an active phase of secretion. The average diameter of the cells is 6.5μ, and that of the nucleus is 4.0μ. Each nucleus has two prominent nucleoli of about 1.2μ in diameter.

As moths get older, the cells become reduced in size and the neurosecretory activity is inhibited. The perikarya of the cells are relatively narrower than those of other moth stages; this is mainly due to the increase in nuclear size and the decrease in the cellular size. In senile condition, the structural details of the nuclei are lost.

3. Type C: The smallest type of neurosecretory cells. During the median larval stages, the average diameters of the cell, nucleus and nucleolus are 4.0μ, 2.6μ and 1.0μ. The perikaryon is narrow (mean width of 1.4μ). The cells contain a moderate amount of cytoplasmic granules. Similar pictures were obtained in the prepupal stages as shown in Table 1 (Figs. 1 & 2).

The neurosecretory activity of the C-cells is somewhat decreased during the pupal stage. The cells show a scant amount of weakly stained neurosecretory material. The cellular, nuclear and nucleolar diameters are approximately 3.2μ, 2.0μ and 0.4μ respectively, and the mean width of the perikaryon is 1.2μ.

In moth stages (including males and females) as well as the early old females, the cells display an active phase of neurosecretory synthesis. The cells have larger cellular and nuclear sizes as compared with those of the pupal stages. The mean diameters of the cells, nuclei and nucleoli are 4.8μ, 3.2μ and 0.7μ respectively. The average width of the perikaryon is 1.6μ.

The ageing C-cells present a gradual regression in their neurosecretory activity. The cells undergo reduction in their cellular, nuclear and nucleolar sizes (Table 1). It is worthy of mention that the C-cells in ageing females retain their neurosecretory activity till a late stage of senility.

Each type of neurosecretory cells in *Spodoptera* presents different pictures in different developmental stages as regards their sizes and phases of neurosecretory activity (Table 2). According to these criteria, each period of the insect's life is characterized by the activity of one or more types of neurosecretory cells in different parts of the central nervous system (Table 3). In the early and median larval stages, the cells of both A- and B- types are present at an active phase of neurosecretion. Such cells are demonstrated in the brain, suboesophageal ganglion, thoracic ganglia and in the abdominal ganglia with the exception of the first, fifth and sixth stages (Fig. 4).

However, the activity of the A- and B-cells in the above-mentioned larval stages may indicate that the secretions produced play a certain role in the growth and moulting of

Table 2
The Relative Activity of the Neurosecretory Cells in the Successive Developmental Stages of Spodoptera littoralis

Stages / *Cell-type*	*EL (3rd age)*	*ML (4th and 5th ages)*	*PP*	*P*	*Am*	*Om*
A	+++	+++	++	+++	++	+
B	+++	+++	+++	+	+++	++
C	++	++	+++	++	+++	+++*

+++ = strong; ++ = moderate; + = weak.
*Active in females only.

Table 3
The Distribution of the Active Neurosecretory Cells in the Nervous Parts of the Successive Stages of Spodoptera littoralis

Stages / *C.N.S.*	*EL (3rd age)*	*ML (4th and 5th ages)*	*PP*	*P*	*Am*	*Om*
Br.	A	A	-	A	AB	-
SO.G	-	A	-	-	-	-
T_1G.	-	B	C	-	-	-
T_2G.	AB	B	BC	A	C	-
T_3G.	AB	-	B	-	-	-
A_1G.	-	-	C	A	B	-
A_2G.	B	B	-	-	B	-
A_3G	A	A	C	A	-	C*
A_4G.	-	B	-	-	BC	C*
A_5G.	-	-	-	-	-	-
A_6G.	-	-	-	-	-	-
A_7G.	-	B	-	-	-	-

*Active in females only.

the larvae. On the other hand, the prepupal stage is generally characterized by a marked activity of secretion of both B- and C- types, particularly those present in the thoracic, and the first and third abdominal ganglia.

It is concluded that these two types of neurosecretory cells might be engaged with the changes which take place in the insect body before pupation. In the pupal stage, only the cells of the A- type show an active phase of neurosecretory synthesis; this may lead to the suggestion that A-cells play a certain role in pupal development. These cells are mostly present in the brain and second thoracic ganglion as well as the first and third abdominal ganglia. In moth stages (males and females) the three types of neurosecretory cells are usually active. These cells are mainly located in the brain; a few are found in the second thoracic, first abdominal, second abdominal and last abdominal ganglia. The synthesis activity in the adult stages may indicate that these secretions have a certain role in the reproductive activities of the insect.

It is worth mentioning that the C-cells continue to show an active phase of neurosecretion in the females till a late stage of ageing – a phenomenon which does not exist in the males of corresponding ages. This may be correlated with the oviposition activity of the females which is known to continue until just before death. Such active cells are found in the third and fourth abdominal ganglia (Table 4).

Inspection of Tables 1 and 2 will show that there is a close correlation between the active phase of neurosecretion on the one hand, and the cellular and nucleolar diameters, as well as the width of the perikaryon, on the other hand. The highly active neurosecretory synthesizing cells have larger cellular and nucleolar diameters and a wider perikaryon than the less actively secreting ones.

Histochemistry of the Neurosecretory Material

Concerning the nature of the neurosecretory material in the different developmental stages, the histochemical data obtained indicate that it includes carbohydrates and proteins. Of the carbohydrates, glycogen, acid mucopolysaccharides and polysaccharide sulphate esters were identified; and of the proteinic materials, histones, tyrosine, tryptophane and sulphur-containing amino acids (cystine and cysteine) were demonstrated. These substances were demonstrated in the neurosecretory cells, as also in the material found in the extracellular and extrafibrillar spaces. Other substances such as phosphatases and ascorbic acid were also demonstrated in the actively synthesizing neurosecretory cells – a finding which indicates that they may play a certain role in the final phases of synthesis, or in the discharge of the neurosecretory material. It was also noticed (Tables 5 and 6) that the neurosecretory material synthesized during the larval and adult stages are mostly proteinic in nature, while those of the pupal stages are rich in carbohydrates. Lipoidal substances were not detected in the neurosecretory material.

Pathways and Transport of the Neurosecretory Material

The neurosecretory materials leave the neurosecretory cells via their axons to the neuropile mass where they either accumulate in the extracellular spaces or become collected in the connecting fibre groups from which the longitudinal connectives extend. From these regions the neurosecretory materials are carried to the different parts of the body where they either exert certain effects, or become transformed into other substances (neurohormones).

Table 4
The Active Neurosecretory Cells During the Body Activities of the Successive Stages of Spodoptera littoralis

Stages	*Body Activity*	*Active Cell-Types*
Larval	Growth and Moulting	A and B
Prepupal	Prepupation	B and C
Pupal	Pupation	A
Adult Moths	Maturation and Reproduction	A, B and C
Ageing Females	Oviposition	C

Table 5
Relative Constitution of the Neurosecretory Material During the Successive Stages of Spodoptera littoralis

	Stages			
Substance	*Larval*	*Pupal*	*Adult Moths*	*Ageing Females*
Carbohydrates	++	+++	++	++
Proteins	+++	++	+++	+++

(++) = small (+++) = rich

Table 6
Histochemical Analysis for the Neurosecretory Material

	Stages						
Inclusions	*EL*	*ML*	*PP*	*P*	*Am*	*Om*	*Sm*
Carbohydrates (PAS)	+	++	+++	++	++	+	-
Glycogen	+	++	+++	++	++	+	-
Mucopolysaccharides	+	++	+++	++	++	+	-
Acid mucopolysaccharides	+	++	+++	++	+	-	-
Polysacch. sulph. est.	+	+++	+++	++	++	-	-
Proteins	++	+++	++	++	+++	++	+
Histones	++	+++	++	++	+++	++	+
Tyrosine	+	+++	++	+	+	-	-
Trytophane	++	+++	++	+	+++	+	+
Cystine	+	+++	++	++	++	-	-
Cysteine	+	+++	++	+	+++	+	-
Ascorbic Acid	++	+++	+++	+++	+++	++	+
Acid phosphatase	+	++	++	++	++	+	-
Alkaline phosphatase	++	+++	+++	+++	+++	++	+

(– absent), (+ traces), (++ moderate), (+++ rich)
This is based on the presence of the neurosecretory materials and their stainability.

As regards the neurosecretory material produced in the brain regions, it may appear that there is a mutual exchange between it and that produced in the corpus allatum-cardiacum complex. This exchange most probably takes place by way of the corpus allatum-cardiacum nerves. However, it is not clear whether the brain material exerts an effect on the cells of the corpus allatum-cardiacum complex or vice versa, since the neurosecretory cells in both organs are usually active at the same time.

The neurosecretory material present in the extracellular spaces is presumably transported by ionic transportation between this material and the haemolymph by what is known as the 'glial lacunar system' [23]. The extrafibrillar spaces demonstrated in the longitudinal connectives of the present material also appear to serve in the transportation of the neurosecretory material, since such material has occasionally been demonstrated within them.

Discussion

The phenomenon of neurosecretion in insects has attracted the attention of a large number of investigators.

According to Hanström [24] and Wigglesworth [25,26], the neurosecretory cells are mainly located in the superficial region of the pars intercerebralis of the protocerebrum of *Rhodnius*. These findings were supported by other authors [27,28,8] in the brains of other insects. Neurosecretory cells were also demonstrated by Delphin [3] in the cortical cellular area of the ventral body ganglia of *Schistocerca*. Later, Banhawy and Anwar [29,4,30,31] reported the presence of neurosecretory cells in the protocerebrum, suboesophageal ganglion, thoracic and abdominal ganglia of *Gryllotalpa*.

In the present study, the neurosecretory cells are located in the forebrain, suboesophageal ganglion, thoracic and abdominal ganglia of the larval stages of *Spodoptera littoralis (B)*. But in moth stages, these cells are found in the forebrain, hindbrain, thoracic and abdominal ganglia.

As regards the neurosecretory cell types, Wigglesworth [32] described in the insect brain one category of large and often lobulated cells which stain deeply with acid fuchsin. Thomsen [33] also recognized in *Calliphora* only one type of neurosecretory cell, besides two other types of cells of unknown function. The latter two types were identified by Thomsen as giant neurones and vacuolated cells. These findings were confirmed by Bloch *et al* [34]. According to Scharrer [35], the neurosecretory cells in the suboesophageal ganglion of *Leucophaea* are of two main types (A and B); a view which has been accepted by Nayar [1], Gangarajah [36], Sainai [37] and Dogra [38]. But Clark [39] and Banhawy and Anwar [4] identified three types of neurosecretory cells (A, B, C) in locusts and *Gryllotalpa* respectively. Delphin [3] recognized in *Schistocerca* four main classes of neurosecretory cells (A, B, C and D), and subdivided type A into A_1, A_2, and A_3, and type B into types B_1 and B_2.

The results obtained from the present investigation are in agreement with those of Clark [39] and Banhawy and Anwar [4]. Each of the three types display different phases of secretory activity in the different developmental stages of the insect. In one phase, the cytoplasm either is empty or contains fine and pale granules. In the second phase, the granules are more conspicuous and are intensely stained. The third phase is that shown by the existence of numerous neurosecretory granules in the cytoplasm,

particularly at the axon pole of the cells.

Highnam [40] considered the empty neurosecretory cells as being active cells, whereas a 'full system' as inactive. This view cannot be accepted by the present authors, who consider the loaded cells as representing an active phase of secretory activity. This finds support in the changes that take place in the Golgi dictyosomes since these are known to play a major role in neurosecretion [41]. Furthermore, Gangarajah [36] reported that the variations in granular sizes from fine granules to clumps suggest a secretory cycle in the insect neurones.

The present study indicates that the active phases of neurosecretions are accompanied by certain variations in the sizes of the cells and their nucleoli, as well as the width of their perikarya. This finds support in the work of Ewen [8], Thomsen [33] and Sainai [37]. On the contrary, Delphin [3] denied the presence of any relation between the cellular activity and the nuclear and nucleolar sizes.

It was also noticed in the present investigation that the active neurosecretory cells are remarkably richer in RNA-inclusions than the non-active ones. This does not agree with Rehm [42], who reported that the elaboration of secretory products is not associated with any increase in the RNA content. On the other hand, the present finding supports those of Pipa [28], Odhiambo [43] and Berry *et al.* [44].

As far as the DNA is concerned, Howells and Birt [45] and Lennie *et al.* [46] noticed a decrease in the DNA in the nuclei of the neurosecretory cells in the early pupae, followed by a rise before emergence. This does not agree with the present finding, which indicates that the nuclear DNA is remarkably increased in the neurosecretory cells during the early pupal stage, whereas emergence is accompanied by an apparent drop in the nuclear DNA. It is also of interest to note that the neurosecretory cells in the latter case have certain DNA-containing elements in their cytoplasm. The presence of DNA in the cytoplasm of the neurosecretory cells was reported for the first time by the present authors [4] in *Gryllotalpa gryllotalpa.*

According to the present results, each period of the insect life is characterized by the activity of one or more types of neurosecretory cells in the different parts of the central nervous system. On the contrary, Delphin [3] stated that only one type of neurosecretory cell (A- type) is obviously active in females during maturation and oviposition activity.

As to the nature of the neurosecretory materials, Rehm [42] and Sloper [47] found that these materials contain a large proportion of proteinic substances which are considered by Sloper [47], Pipa [28], Wigglesworth [48], Adams [49] and Dogra [38] to be rich in cystine and/or cysteine. Schiebler [50, 51] reported that the proteins exist in the form of glycolipoprotein complex. However, Banhawy and Anwar [4] demonstrated in the neurosecretory cells of *Gryllotalpa* a proteinic substance rich in histones, cystine and cysteine, but the lipoidal material is lacking. Also, no lipids were detected in the present material. The absence of lipids in the neurosecretory material was similarly reported by Sloper [52] and Howe and Pearse [53].

As regards the mechanism of the neurosecretory synthesis, it is clear that the *Golgi dictyosomes* play a major role in this respect. This is based on the correlation between the mode of occurrence of the *Golgi* elements and the presence of secretory granules in the different cells. [See also 41 and 29.]

Concerning the transport of the neurosecretory materials, several authors believe that

these materials are transported from the protocerebrum along the axons where they are stored in the corpus cardiacum or in corpus allatum [54, 55, 56, 35, 57, 58, 59, 60]. Other authors consider that the neurosecretory material of the protocerebrum is discharged into the aortic lumen to the corpus allatum, where it diffuses into the haemolymph via the membrane of the corpus allatum. This view was not accepted by Herlant-Meewis and Paquet [61], Naisse [62] and Wigglesworth [63]. On the other hand, Ewen [64], Johansen [65] and Banhawy and Anwar [29] are of the opinion that the aorta is the principal means of transportation of the neurosecretory material between the brain and corpus allatum and corpus cardiacum in both directions.

The present results indicate that the neurosecretory material is transported from/to the brain and corpus allatum-cardiacum complex via the corpus allatum-cardiacum nerves. The neurosecretory material present in the extracellular spaces is probably transported into the haemolymph by what is known as glial lacunar system as has been previously described by Wigglesworth [23] and Treherne [66]. The extrafibrillar spaces demonstrated in the longitudinal connectives and peripheral nerves of the present material appear to serve in the transportation of neurosecretory substances to the different body regions.

Summary

1. Three types of neurosecretory cells (A-, B-, and C- types) exist in the cotton leaf worm, *Spodoptera littoralis,* in the different parts of the nervous system with the exception of the frontal ganglion.
2. The neurosecretory cells represent different phases of activity in the various parts of the nervous system of the different developmental stages of the insect.
3. The neurosecretory cells and the neurosecretory material contain:
   (a) polysaccharides including glycogen, acid mucopolysaccharides and polysaccharide sulphate esters;
   (b) proteins, in the form of histones, tyrosine, tryptophane, SS- and SH-containing groups;
   (c) phosphatases, and
   (d) ascorbic acid.

Lipids are lacking.
4. The neurosecretory material leaves the neurosecretory cells via their axons to the neuropile mass where it either accumulates in the extracellular spaces or becomes collected in the connecting fibre groups from which the longitudinal connectives extend.

REFERENCES

1. K. Nayar, 'Studies on the neurosecretory system of *Iphita limbata* (stal.)', Biol. Bull. Wood's Hole, *108,* 296-307 (1955).
2. A. Johannson, 1958, quoted in 'Histological and histochemical studies on the insect nervous system, *Spodoptera littoralis (B)',* Ph. D. thesis, Assiut University, U.A.E., 1971.
3. F. Delphin, 'The histology and possible functions of neurosecretory cells in the ventral ganglia of *Schistocerca gregaria',* Trans. Roy. Ent. Soc., *117,* 167-214 (1965).
4. M. Banhawy & I. Anwar, 'The neurosecretory cycle and neurosecretory substance in *Gryllotalpa gryllotalpa (L)',* Ann. Zool., *7,* 42-63 (1971).
5. N. Halmi, 'Differentiation of two types of basophils in the adenohypophysis of the rat and the mouse', Stain Tech., 27-61 (1952).
6. F. Delphin, 'A new differentiatial staining technique for neurosecretory substances', J. Life Sci., *1,* 26-7 (1968).
7. C. Pantin, *Notes on Microscopical Technique for Zoologists,* Cambridge Univ. Press (1962).
8. A. Ewen, 'An improved aldehyde fuchsin staining technique for neurosecretory products in insects', Trans. Amer. Micr. Soc., *81,* 94-6 (1962).
9. R. Hotchkiss, Arch. Biochem., *16,* 131 (1948).
10. L. Lison, 'A technique for selective staining of mucopolysaccharides', Stain Tech., *29,* 131-8 (1957).
11. C. Hale, 'Histochemical demonstration of acid polysaccharides in animal tissue', Nature, *157,* 802 (1946).
12. H. Kiyoshi, 'The histochemical significance of staining polysaccharide sulphate esters with gentian violet', Stain Tech., *31,* 71-5 (1956).
13. A. Pearse, *Histochemistry (Theoretical and Applied),* J. & A. Churchill Ltd., London (1961).
14. W. Schneider, 1945, quoted in [13].
15. R. Bensley and I. Gersh, Anat. Rec., *57,* 369-84 (1933).
16. E. Gurr, *Methods of Analytical Histology and Histochemistry,* Leonard Hill Ltd., London (1958).
17. J. Gatenby and T. Moussa, 'The sympathetic ganglion cell with sudan black and Zernicke microscope', J. Roy. Micr. Soc., *70,* 342-64 (1950).
18. N. Kurnick, 'Histochemistry of nucleic acids', Stain Tech., *30,* 213 (1955).
19. G. Bourne, 1935, quoted in [13].
20. S. Barnett and G. Bourne, 'Use of silver nitrate for the histochemical demonstration of ascorbic acid', Nature, *147,* 542-3 (1941).
21. G. Gomori, 'Acid phosphatase technique', Stain Tech. *25,* 81 (1950).
22. G. Gomori, *Microscopic Histochemistry – Principles and Practice,* Chicago Univ. Press (1953).
23. V. Wigglesworth, 'The nutrition of C.N.S. in the cockroach, *Periplaneta americana (L)',* J. Exp. Biol., *37,* 500-12 (1960).
24. B. Hanström, 'Neurosecretory cells: (Rhodnius)', Lunds. Univ. Arsskr. N. F., *34,* 1-17 (1938).
25. V. Wigglesworth, 'Source of the moulting hormone in *Rhodnius',* Nature, *144,* 753 (1939).
26. V. Wigglesworth, 'The determination of characters at metamorphosis in *Rhodnius prolixus',* J. Exp. Biol., *17,* 201-22 (1940).
27. M. Rehm, 1951, quoted in *Advances in Insect Physiology* by V. B. Wigglesworth, Methuen, London.
28. R. Pipa, 'Studies on the hexapod nervous system', Biol. Bull., *121,* 521-34 (1961).
29. M. Banhawy and I. Anwar, 'Morphology and histology of the brain of *Gryllotalpa gryllotalpa (L)',* Ann. Zool., *6,* 141-51 (1970).

30. M. Banhawy and I. Anwar, 'A histological study of the suboesophageal ganglion of *Gryllotalpa gryllotalpa (L)* with special reference to the localization of the neurosecretory cells', Bull. Ent. Soc. Egypt, *56,* 419-28 (1972).
31. M. Banhawy and I. Anwar, 'Histological studies on the thoracic ganglia of the nymphal and adult stages of *Gryllotalpa gryllotalpa (L)* in relation to their functional significance', Bull. Ent. Soc. Egypt, *56,* 389-98 (1972).
32. V. Wigglesworth, *The Nervous System (Principles of Insect Physiology),* Methuen, London, 114-28 (1953).
33. M. Thomsen, 'The neurosecretory system of the adult *Calliphora erythrocephala',* Zeit. Zellf., *67,* 693-717 (1965).
34. B. Bloch, E. Thomsen and M. Thomsen, 'The neurosecretory system of the adult *Calliphora erythrocephala',* Zeit. Zellf., *70,* 185-208 (1966).
35. B. Scharrer, 'Aberrations in the distribution of neurosecretory cells in the suboesophageal ganglion of *Leucophaea maderae',* Anat. Rec., *122,* 389-490 (1955).
36. M. Gangarajah, 'The neuro-endocrine complex of adult *Nebria brevicollis (F)* and its relation to reproduction', J. Insect Physiol., *11,* 1377-87 (1965).
37. R. Sainai, 'Neuro-endocrine control of Oocyte development of the beetle *Hulacophora foveicollis Luc.',* J. Insect Physiol., *12,* 1003-8 (1966).
38. G. Dogra, 'Studies on the neurosecretory system and the functional significance of NSM in the aortal wall of the bug, *Dysdercus koenigii',* J. Insect Physiol., *13,* 1895-906 (1967).
39. U. Clark, 'Histological changes in the endocrine system of *Locusta migratoria L.* associated with the growth of the adult under different temperature regions', J. Insect Physiol., *12,* 163-70 (1966).
40. K. Highnam, Zool. Jahrb. Abt. Physiol., *71,* 558-82 (1965). Quoted in [67].
41. T. Moussa and M. Banhawy, 'The Golgi dictyosomes during the differentiation and growth of the nerve cells of *Schistocerca gregaria* with special reference to the problem of neurosecretion', J. Roy. Micr. Soc., *79,* 19-36 (1959).
42. M. Rehm, 'Morphologische und histologische Untersuchungen an neurosekretorischen Zellen von Schmetterlingen', Z. Zellf., *42,* 19-58 (1955).
43. T. Odhiambo, 'Ultrastructure of the development of the corpus allatum in the adult male of the desert locust', J. Insect Physiol., *12,* 995-1002 (1966).
44. S. Berry, A. Krishnakumeran, H. Oberländer and H. Schneiderman, 'Effect of hormones and injury on RNA synthesis in saturniid moths', J. Insect Physiol., *13,* 1511-37 (1967).
45. A. Howells and L. Birt, 'Amino acid dependent pyrophosphate exchange during the life cycle of the blowfly *Lucilla cuprina',* Comp. Biochem. Physiol., *11,* 61-83 (1964).
46. R. Lennie, D. Gregory and L. Birt, 'Changes in the nucleic acid content and structure of thoracic mitochondria during development of the blowfly, *Lucilla cuprina',* J. Insect Physiol., *13,* 1745-56 (1967).
47. J. Sloper, 'Presence of a substance rich in protein-bound cystine or cysteine in the neurosecretory system of an insect', Nature, *179,* 148-9 (1957).
48. V. Wigglesworth, 'The action of moulting hormone and juvenile hormone at the cellular level in *Rhodnius prolixus',* J. Exp. Biol., *40,* 231-45 (1963).
49. C. Adams, 'Neurosecretion', *Neurohistochemistry,* Elsevier, Amsterdam, London, N. Y., 309-19 (1965).
50. T. Schiebler, 1951, quoted in [49].
51. T. Schiebler, Exptl. Cell Res., *3,* 249-50 (1952). Quoted in [67].
52. J. Sloper, 'Hypothalamic neurosecretion in the dog and cat with particular reference to the identification of neurosecretory material with posterior lobe hormone', J. Anat., *89,* 301-16 (1955).
53. A. Howe and A. Pearse, 'A histochemical investigation of neurosecretory substance in the rat', J. Histochem. Cytochem., *4,* 561-9 (1956).

54. B. Hanström, 'Inkretorsche Orange, Sinnesorgane und Nervensystem des Kopfes einiger nieder Insektordnungen,' K. Svenska vetensk. Akad. Handl. Zser., 18-226 (1940).
55. L. Arvy, 'Données histophysiologique sur la neuro-sécrétion chez quelques Ephéméroptères', La Cellule, *55,* 203-24 (1953).
56. L. Arvy and M. Gabe, 'Modifications de la neurosécrétion protocerebrales et des glandes endocrines cephaliques de *Leptinotarsa decemlineata* Say au cours de la métamorphose', 79^{e} Congr. Soc. Savantes, 189-96 (1954).
57. B. Scharrer, 'The fine structure of the neurosecretory system of the insect *Leucophaeae maderae',* Mem. Soc. Endocrin., *12,* 89-97 (1962).
58. V. Wigglesworth, 'The haemocytes and connective tissue formation in an insect, *Rhodnius prolixus',* Quart. J. Micr. Sci., *97,* 88-98 (1956).
59. K. Highnam, 'Induced changes in the amounts of material in the neurosecretory system of the desert locust', Nature, *191,* 199-200 (1961).
60. K. Highnam, 'The histology of the neurosecretory system of the adult female desert locust *Schistocerca gregaria',* Quart. J. Micr. Sci., *1, 102,* 27-38 (1961).
61. M. Herlant-Meewis and L. Paquet, 'Neurosécrétions et mue chez *Carausius morus',* Ann. Sci. Nat. (Zool.), *18,* 163-9 (1956).
62. J. Naisse, 'Neurosecretion and corpora cardiaca-corpora allata during post-emb. development in *Lampysis',* J. Comp. End., *2,* 630-1 (1962).
63. V. Wigglesworth, 'Advances in Insect Physiology', *2* (1964).
64. A. Ewen, 1962.
65. B. Johansen, 'Neurosecretion and the transport of secretory material from the corpora cardiaca in aphids', Nature, *196,* 1338-9 (1962).
66. J. Treherne, 'The neurochemistry of arthropods', Cambridge Monographs in Experimental Biology no. 14 (1966).
67. I. Anwar and S. Ismail, 'Neurosecretory centres in the brain of adult *Gryllus bimaculatus D',* Int. J. Insect Morphol. and Embryol., *8* (1979).