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A HISTOLOGICAL STUDY ON THE NEUROENDOCRINAL SYSTEM OF A COLEOPTEROUS INSECT, CYBISTER TRIPUNCTATUS AFRICANUS CAST.

By

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ABSTRACT

Four different types of neurosecretory cells were identified in the brain of the adult water beetle, *Cybister tripunctatus africanus Cast.* These cells were identified into A, B, C, & D types according to their morphological features and histochemical affinities.

These cells were concentrated in three main locations in the brain, median portion of protocerebrum, lateral region of protocerebrum, and tritocerebrum. Thus, three endocrinal centers (or glands) are present in the brain of the adult insect.

Certain differences were observed in the neurosecretory activity of these cells in the brains of male amd female insects.

Ten phases of activities are displayed by the four of neurosecretory cells in the three different locations in the brain.

INTRODUCTION

Since Hanstrom (1938, 1940), several authors have reported the presence of neurosecretory cells in the brain and other nerve ganglia of insects. Many investigators regarded those neurosecretory cells as endocrinal centers of different functions (Van der Klott, 1960; Highnam, 1961; Wigglesworth, 1964; Thomsen, 1965; Novak, 1966; Banhawy & Anwar, 1970; 1972 a, b; 1973; 1980; and Anwar & Ismail, 1979). The distribution of those cells in the tissue of the central nervous system and other associated nerve ganglia has been the field of interest by numerous authors who investigated a wide range of insect species (Moussa & Banhawy, 1958 on *Schistocerca gregaria;* Ewen, 1962 and Khattar, 1968 on *Shizodactylus menstrosus;* Banhawy & Anwar, 1970, 1972 a, b, 1973, 1980 on *Gryllotalpa gryllotalpa* and *Spodoptera littoralis*; Geldiay & Edwards, 1973 on *Acheta domasticus;* Panov, 1976 on *Calliphora vicina;* Charlet & Schaller, 1977 on *Aeshne cyanes;* and Anwar & Ismail, 1979 on *Gryllus bimaculatus*).

Further, several authors were particularly interested in the investigation of the different types of those neurosecretory cells depending on the location of each type of those cells and application of specific histochemical techniques (Dupont-Raabe, 1956; Highnam, 1961; Girardie & Girardie, 1967, 1972; Baehr, 1969; and Banhawy & Anwar, 1971, 1980; Geldiay & Edwards, 1973; Highnam, 1976; Panov, 1978; and Anwar & Ismail, 1979).

According to Moussa and Banhawy (1958) and Odhiambo (1966) the Golgi elements (dictyosomes) of the insect nerve cells undergo marked changes which accompany the insect's development as well as during cellular activity. Furthermore, Banhawy & Anwar (1970) concluded that the Golgi elements played an active role in the secretory activity of the nerve cells and that the morphology and topography of those elements differed in secretory cells comparable to the non-secretory ones.

The chemical nature of the neurosecretory material was discussed by few authors in certain insects (Banhawy & Anwar, 1971, 1980; Prento, 1972; and Anwar & Ismail, 1979).

The aim of the present work is to give an account of the histology and histochemistry of the neurosecretory centers in the brain of the adult water beetle, *Cybister tripunctatus africanus* Cast; at different intervals following emergence of that adult.

MATERIALS AND TECHNIQUES

Cybister tripunctatus africanus Cast, the sommon water beetle in Egypt, was found in great numbers, in the fresh water bonds of Abou-Roach in Giza province. Adult beetles could be safely kept together in one aquarium. Lettuce, cabbage leaves maize leaves, snails and annelides were given, as food materials, for this omnivorous beetle.

The female insect was never observed to oviposit in captivity. However, adult females layed their eggs during the month of February and died after a short time. The new winged generation appeared in November. During the period between February and November adult insects are very rare in the field.

Dissection was carried out in Ringer's solution. For the general histological examinations, fixation be aqueous Bouin and staining by Heidenhain's azan stain gave very good results. For the detailed study of the neurosecretory cells, Susa fixative and three staining techniques including paraldehyde fuchsin and its modification recommended by Ewen (1962). Heidenhain's azan stain, and haematoxylin chrome phloxine of Gomori (1953) were very satisfactory and best served the target needed.

For the demonstration of carbohydrates, whether qualitatively, or relatively quantitatively (according to the depth of the red colour), PAS test was applied. In such a case the material, undertest, was fixed in Carnoy's Fluid.

For the demonstration of acid mucopolysaccharides, material was first fixed in Bouin's or Carnoy's solutions than stating was accomplished by using a fluid compacted of equal volumes of 1% aqueous alcian blue and acetic acid as recommended by Lison (1957). Haematoxylin was used as a counterstain. Application of a bluish-green colour referred to the presence of acid mucopolysaccharides.

Acidic proteins were illustrated in material fixed by Bouin's solution, stained by the use of 0.1% toluidine blue (Kramer & Windrum, 1954). Acidic proteins gave a blue colour.

To demonstrate basic proteins, material was fixed in Bouin's fluid, and stating was carried out in 0.1% fast green as recommended by Pearse (1968). A green colour indicated the presence of basic proteins.

Liquids were displayed in the present material by the technique adopted by Gatenby and Moussa (1950). For this purpose, material was first in Elftman's fluid then stained by Sudan black 8. The appearance of a blue-black colouration indicated the presence of lipids.

For the demonstration of Golgi elements, Da Fano (1920) technique was adopted and it gave good results. The Golgi elements appeared as brownish bodies scattered in the cytoplasm.

RESULTS AND DISCUSSION

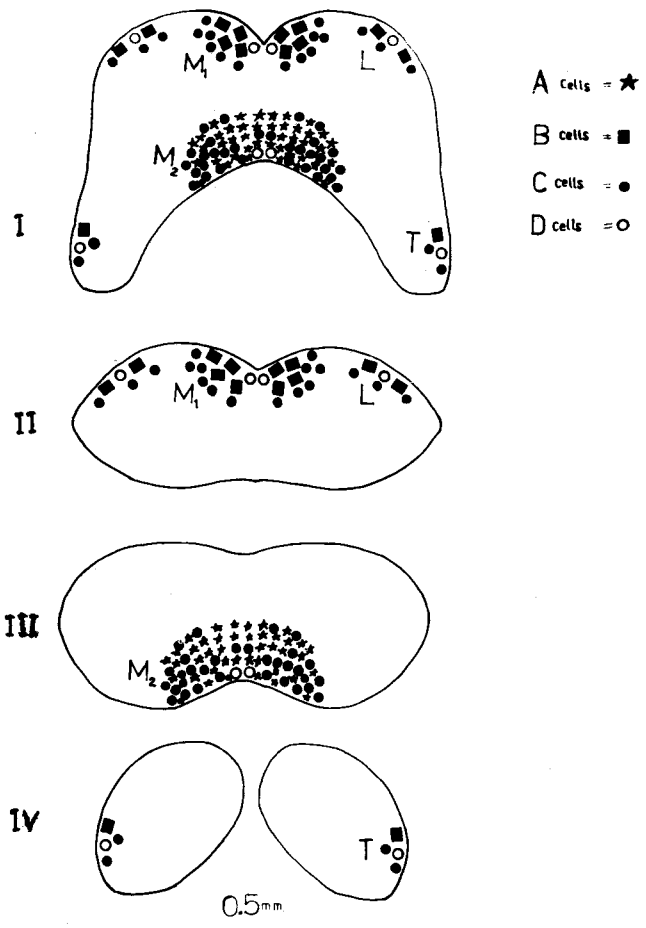
The neurosecretory cells were identified a long time ago as endocrinal unicellular glands which secrete hormones responsible for the internal chemical coordination (Wigglesworth, 1940, 1953, 1959 & 1964; Highnam, 1961; Thomsen, 1965; Anwar & Ismail, 1979 and Banhawy & Anwar, 1980. In the present *Cybister tripunctatus africanus* Cast., the different types of neurosecretory cells (fourtypes: A, B, C, D, which will be described later) were found to be present in certain location of the brain. Those locations are; median portion of protocerbrum, lateral region of protocerebrum, and tritocerebrum which are presented in the Figures 1, II, III, IV, and Table 1 which shown also their average numbers in both males and females. A similar mode of distribution of neurosecretory cells in the insect's brain was recorded in *Locusta migratoria* by Girardie & Girardie (1967); in *Panostronglus megistus* by Furtado (1976); in *Gryllus bimaculatus* by Anwar & Ismail (1979); and in *Spodoptera littoralis* by Banhawy & Anwar (1980)

Table 1

Distribution of different types of neurosecretory cells in the cerebral neurosecretory centres of adult, *Cybister tripunctatus africanus* (Cast).

Neurosecretory cells centres	Types of the cells				
	Sex	A	B	C	D
Anterior median NSC	♀	—	20	56±1	6
	♂	—	24±2	62±2	6
Posterior median NSC	♀	218±2	—	155±2	2
	♂	231±1	—	162±2	2
Lateral NSC	♀	—	8±2	17±1	4
	♂	—	9±1	10±1	4
Tritocerebral NSC	♀	—	6	9	2
	♂	—	6±2	9	2

NSC = Neuosecretory cells



Figures 1 to 5. Diagrammatic representations of the distribution of the four types A. B. C. and D - neurosecretory cells in the three locations, median (M1 & M2), lateral (L), and tritocerebral (T).

All the four types of neurosecretory cells mentioned in Table 1 were stained by three different techniques, namely paraldehyde Fuchsin, Heidenhain's azan and Gomori's chrome haematoxylin phioxin (plate I, figs. 1 to 11). But different specific histochemical techniques were applied to differentiate these various types (plates II & III, Figures 12 to 31). The results of those staining and histochemical experiments are illustrated in Tables (2 & 3). Those reactions were carried out with the aim to prove that the different response of each type of cell towards a certain technique could be an indicator that the type of cells, under test, was a real independent and genuine type.

Table 2
Histological reactions of A, B, C and D neurosecretory cells

Types	Cell diameter	Nuclear diameter	Histological reactions		
			Ewen's paraldehyde Fuchsin	Heidenhain's azan	Gomori's chrome-haematoxylin
A	18-20 u	11-13 u	deep purple Pl. 1, Fig. 1	red Pl. 1, Fig. 2	dark blue Pl. 1, Fig. 3.
B	20-25 u	12-15 u	violet green Pl. 1, Fig. 4.	violet red Pl. 1, Fig. 5.	red Pl. 1, Fig. 6.
C	18-20 u	11-14 u	violet Pl. 1, Fig. 7.	orange red Pl. 1, Fig. 8.	mauve Pl. 1, Fig. 9.
D	38-44 u	16-20 u	faint violet Pl. 1, Fig. 1.	mauve Pl. 1, Fig. 10.	mauve Pl. 1, Fig. 11.

Certain attempts have been made by few investigators to clarify the chemical nature of the neurosecretory materials of one or more neurosecretory cell type in insects (Schrainer, 1966; Girardie & Girardie, 1967; Banhawy & Anwar, 1971; Prento, 1972; Anwar & Ismail, 1979; and Banhawy & Anwar, 1980).

It should be noticed that the above mentioned staining affinities and histochemical characteristics of the different types of neurosecretory cells (Tables 2 & 3) were true for young male and female images of *Cybister tripunctatus africanus* whose ages were 65 - 80 days after emregence. In older images, the cell reactions faded and decreased in degree to the extent that they could not be relied upon in the demonstration and identificattions of these different cell types.

The state of ativity of a neurosecretory cell was indicated by the accumulation of the secretory granules, in a very dense form, round the nucleus. The cell could be described in this as a loaded cell (Bnhawy & Anwar, 1971, 1980; and Anwar & Ismail, 1979). Tables 3 to 6 illustrate the percentage of the number of located cells relative to the total number of cells of the same type in each location, and for each sex at four dates (20, 50, 80 & 110 days) following emergence.

Examination of table shows the presence of only one location for A-type (in the median region of the brain); and three locations (median, lateral and tritocerebral) for each of the three types of neurosecretory cells (B, C, & D). The sites proposed for these cells are Am or (A-median), Bm or (B-median), Bl or (B-lateral), Bt or (B-tritocerebral), Cm, Cl, Ct, Dm, Dl and Dt. It is clear from this description that ten phases of activities are displayed by the four types of neurosecretory cells in the three different location of the brain. Examination of those tables (3 to 6) also show that the percentage of activity of the cell types - B, C or D was dependent on the sex as well as their localization. The dependence of that phenomenon on sex is quite

Table 3

. Histochemical reactions of A, B, C, and D neurosecretory cells

Types	Histochemical reactions					
	Acidic proteins	**Basic proteins**	**Carbohydrates**	**Acid mucopoly-saccharides**	**Lipids**	**Golgi elements**
	Toluidine blue	**Fast green**	**P.A.S.**	**Alcain blue**	**Sudan black B**	**Da Fano**
A	moderate Pl. II, Fig. 12.	moderate Pl. II, Fig. 13.	moderate Pl. II, Fig. 14.	high Pl. III, Fig. 22.	high Pl. III, Fig. 23.	high Pl. III, Fig. 24.
B	high Pl. II, Fig. 15.	high Pl. II, Fig. 16.	high Pl. II, Fig. 17.	moderate Pl. III, Fig. 25.	high Pl. III, Fig. 26.	high Pl. III, Fig. 24.
C	high Pl. II, Fig. 18.	moderate Pl. II, Fig. 13.	traces Pl. II, Fig. 19.	moderate Pl. III, Fig. 25.	moderate Pl. III, Fig. 27.	high Pl. III, Fig. 28.
D	moderate Pl. II, Fig. 12.	high Pl. II, Fig. 20.	moderate Pl. II, Fig. 21.	moderate Pl. III, Fig. 29.	high Pl. III, Fig. 30.	moderate Pl. III, Fig. 31.

Table 4

The degree of accumulation of neurosecretory materials in different types of NSC in the adult brain.
(20 days after adult emergence)

Types		MNSC ♂	♀	LNSC ♂	♀	TNSC ♂	♀
A	Loaded	110±5	145±3				
	Total	230±1	214±4				
	%	47.8	67.7				
B	Loaded	11±2	16±1	3±1	3±1	3	3
	Total	24±1	20	8±2	6±3	6±2	6±1
	%	45.8	80	37.5	50	50	50
C	Loaded	95±2	100±3	3	6±2	3	3
	Total	220±3	212±4	9±1	17±1	9	9
	%	43.1	47.1	33.3	35.2	33.3	33.3
D	Loaded	3±1	3±2	1	3	1	1
	Total	8	8	4	4	2	2
	%	37.4	37.4	25	75	50	50

MNSC. — Median neurosecretory cells
TNSC. — Tritocerebral neurosecretory cells
LNSC — Lateral neurosecretor cells
% — The percentage of active cells

Table 5

The degree of accumulation of neurosecretory materials in different types of NSC in the adult brain
(50 days after adult emergence)

Types		MNSC ♂	♀	LNSC ♂	♀	TNSC ♂	♀
A	Loaded	128±1	170±2				
	Total	230±3	218±1				
	%	55.6	77.7				
B	Loaded	13±1	16±1	4±2	4±1	3±1	4±2
	Total	24±1	20	8±1	8±2	6±2	6±2
	%	54.1	80	50	50	50	66.6
C	Loaded	100±3	95±2	5±1	7±1	4	3
	Total	224±3	210±5	10±4	16±1	9±2	9
	%	45.09	45.2	50	43.7	44.4	33.3
D	Loaded	4±1	4	2±1	3	1	2
	Total	8	8	4	4	2	2
		50	50	50	75	50	100

Table 6

The degree of accumulation of neurosecretory materials in different types of NSC in the adult brain

(80 days after adult emergence)

Types		MNSC ♂	♀	LNSC ♂	♀	TNSC ♂	♀
A	Loaded	143±2	1801				
	Total	233±2	220±1				
	%	61.4	81.8				
B	Loaded	17±2	18±1	6±2	7±1	3±1	4±2
	Total	24±3	10	10±3	10±1	8±2	6±1
	%	70.8	90	60	70	37.5	66.6
C	Loaded	100±3	115±2	6±1	9±1	5±2	4±2
	Total	222±5	209±5	11±1	17±2	9±1	9±1
	%	45.04	55.5	54.5	52.9	55.5	44.4
D	Loaded	7±1	7±2	3	3	2±1	2
	Total	8	8±1	4	4±1	2	2±2
	%	87.5	87.5	75	75	100	100

understandable (Ewen, 1962 and Anwar & Ismail, 1979), but its dependence on localization necessitates a special detailed study. These observations are generally in confirmation with the findings obtained by Banhawy and Anwar (1971 & 1980) in *Gryllotalpa gryllotalpa* and *Spodoptera littoralis* and by Anwar and Ismail 1979) on *Gryllus bimaculatus*. According o these authors, the neurosecretory cell types show different pictures of the cellular activity during their neurosecretory cycle in the brain of insects.

Histochemically, each group of neurosecretory cell-types, present in a certain location in the brain of a certain sex (male or female) and same age reveals collectively the same histochemical patterns of reactions. This observation leads to the suggestion that each such groups form an endocrine centre (gland) which secretes one of more hormones.

Table 7

The degree of accumulation of neurosecretory materials in different types of NSC in the adult brain

(110 days after adult emergence)

Types		MNSC ♂	♀	LNSC ♂	♀	TNSC ♂	♀
A	Loaded	140±1	91±1				
	Total	232±1	220±4				
	%	60.3	41.3				
B	Loaded	11±1	5±2	3	3±1	3±1	3
	Total	24±2	20	10±3	8±2	6±1	6±1
	%	45.8	25	30	37.5	50	50
C	Loaded	55±1	25±2	3±2	8±1	4±2	3
	Total	226±4	211±5	10±2	17±1	9±1	9±2
	%	24.3	11.8	30	47.04	44.4	33.3
D	Loaded	4±1	4±1	0	0	0	0
	Total	8±2	8±1	4±1	4±2	2	2
	%	50	50	0.0	0.0	0.0	0.0

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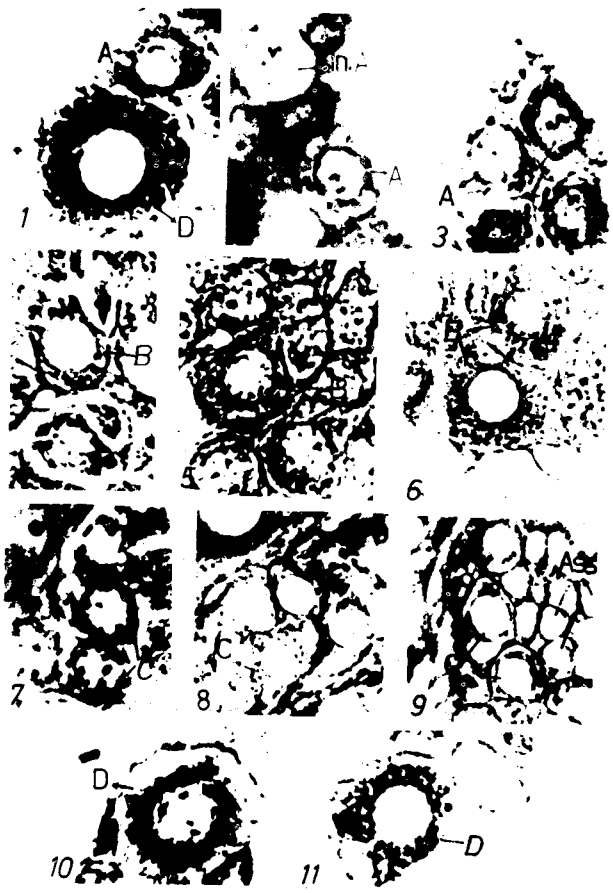

PLATE I:
All figures at magnification × 800.

Figure 1-3: A-type of neurosecretory cells, stained by Ewen in Figure (1), by Heidenhain's azan in Figure (2), and Gomori in Figure (3). In Figure (1) notice the largest D-type cell, in Figure (2) the lower A-cell is isolated while the upper is unloaded (in A).

Figures 4-6: 8-types of neurosecretory cells, stained by Ewen in Figure (4), by Heidenhain's azan in Figure (5), and Gomori in Figure (6).

Figures 7-9 C-type neurosecretory cells, stained by Ewen in Figure (7), by Heidenhain's azan in Figures (8) and Gomori in Figure (9). Notice the presence of few association (Ass) non-neurosecretory cells.

Figure 10-11. D-type of neurosecretory cells, stained by Heidenhain's azan in Figure (1), and Gomori in Figure (11).

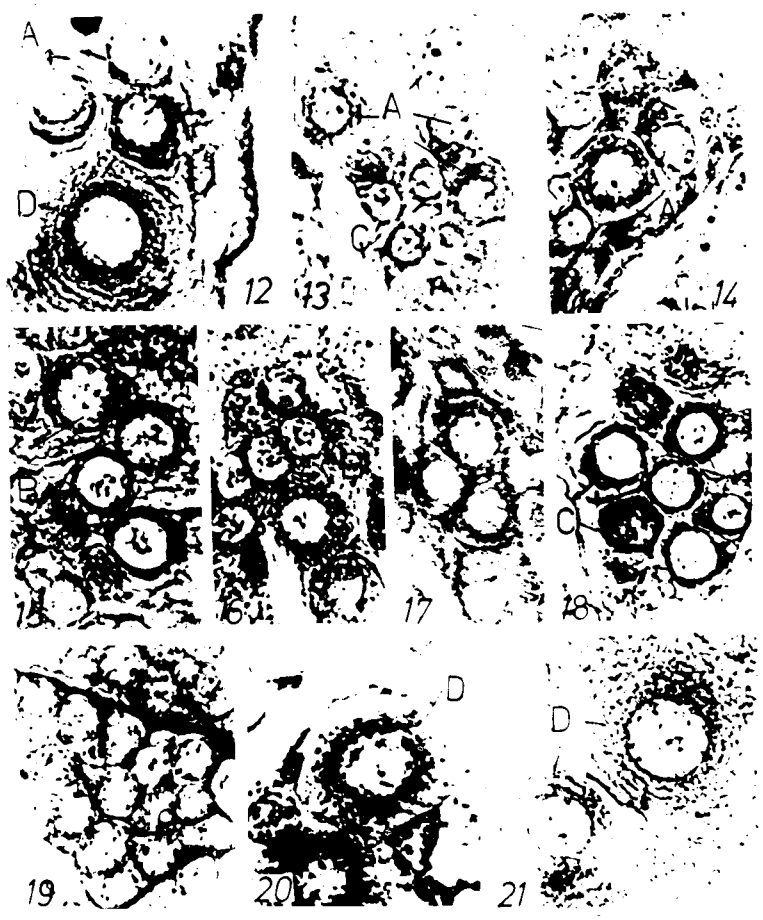

PLATE II
All figures at magnification × 640

Figures 12-14. A-type of neurosecretory cells- stained by Toluidine blue in figure (12), by Fast green in Figure (13), and P.A.S. in Figure (14). Notice d-type cell in Figure (12), and C-type cell in Figure (13).

Figures 15-17. B-type of neurosecretory cells, stained by Toluidine blue in Figure (15), by Fast green in Figure (16), and PAS in Figure (17).

Figures 18-19. C-type of neurosecretory cells, stained by Toluidine blue in Figure (18), and PAS in Figure (19).

Figure 20-21. D-type of neurosecretory cells, stained by Fast green in Figure (20), and PAS in Figure (21).

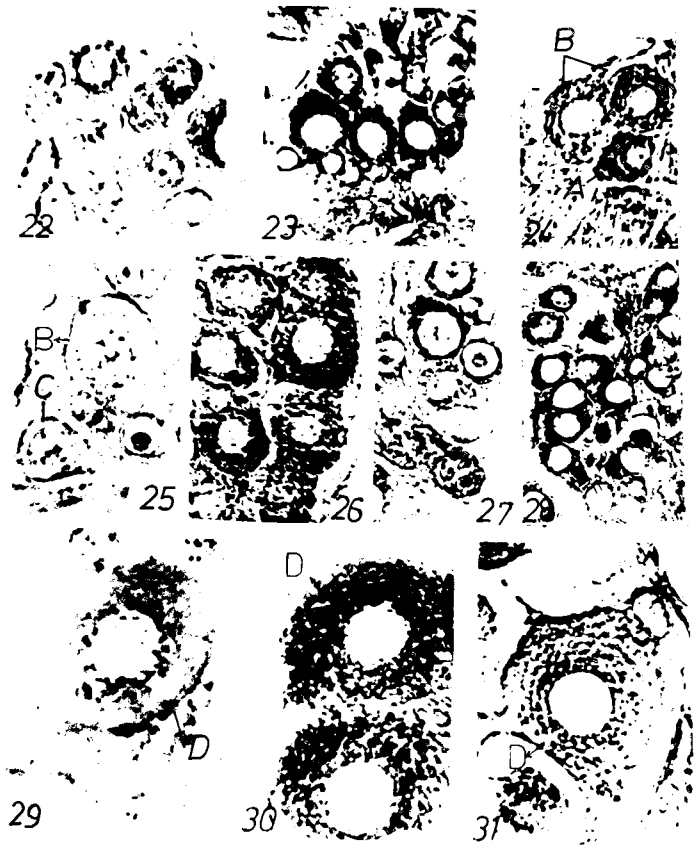

PLATE III
All Figures at magnification × 640

Figure 22-24. A-type of neurosecretory cells, stained by Alcian blue in Figure (22), by Sudan black in figure (23), and Da Fano in Figure (24). Notice B-type cells in Figure (22).

Figure 25-26. B-type of neurosecretory cells, stained by Alcian blue in Figure (25), and Sudan Black in Figure (26). Notice C-type cell in Figure (25).

Figure 27-28. C-type of neurosecretory cells, stained by Sudan black in Figure (27), and Da Fano in Figure (28).

Figure 29-31. D-type of neurosecretory cells, stained by Alcian blue in Figure (29), by Sudan black in Figure (30), and Da Fano in Figure (31).

هذا بالاضافة إلى أنه من وجهة النظر الهستوكيميائية يمكن تحديد وجود ثلاثة مراكز افرازية (أو غدية) في المخ للطور الكامل للحشرة حيث يحتوى كل مركز منها على أكثر من نوع من الخلايا الافرازية التي تشترك في افراز هرمون واحد أو أكثر .

هستوفسيولوجية على جهاز الأفراز العصبي في حشرة غمديه الأجنحة « سيبستر ترايبنكتاتس أفريكانس كاست »

إبراهيم محمد أنور

ملخص

تهدف الدراسة الحالية إلى القاء مزيد من الضوء على هستولوجية وهستوكيميائية المراكز الافرازية الثلاثة (الوسيطة ، الجانبية ، الخلفية) المكونة لجهاز الافراز العصبي في مخ الطور الكامل للخنفساء المائية (سيبستر ترايبنكتاتس افريكانس كاست) على فترات متتابعة بعد نضج الطور الكامل في كل من الذكر والأنثى .

وقد تم تمييز أربعة أنواع من الخلايا العصبية الافرازية (أ ، ب ، جـ ، د) طبقاً للصفات المورفولوجية للخلايا ومدى قابليتها للصباغة بالصبغات الهستولوجية والهستوكيميائية الخاصة .

وأمكن تحديد اعداد خلايا كل نوع من الخلايا الافرازية الموجودة في كل مركز من مراكز الافراز العصبي بالمخ ، كذلك تم ايجاد النسب المئوية للخلايا النشطة بالنسبة للعدد الكلي لكل نوع في كل مركز افرازي . وقد تميزت الخلايا النشطة باحتوائها على كمية كبيرة من المواد الافرازية ذات القابلية الشديدة للصبغات المميزة لمثل هذه المحتويات الافرازية ، وذلك على النقيض من الخلايا الاقل نشاطاً حيث تنخفض قابليتها للصباغة إلى حد كبير .

وقد اتضح مما سبق اختلاف نشاط خلايا النوع الواحد من مكان إلى آخر في المخ للعمر الواحد لنفس الجنس (ذكر أو أنثى) .. ومن ذلك أمكن ، فسيولوجيا ، تمييز عشرة صور لمراحل النشاط الافرازي للأربعة أنواع من الخلايا الافرازية موزعة في مراكز الافراز العصبي الثلاثة (الوسيطة ، الجانبية ، الخلفية) بالمخ .