CHAPTER-III

OBSERVATIONS

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1 *SEMPERULA MACULATA*

A) *HISTLOGICAL OBSERVATIONS ON CEREBRAL GANGLION DURING BREEDING SEASON* :

The histological data on some important histological staining techniques employed in the present investigation of cerebral ganglion in the breeding season are recorded in Table No.1, according to the visually estimated intensity and shade with four plus (++++) representing the strongest activity. The histological observations requiring further description and consideration are presented hereafter. The histological distribution of various neurosecretory cells in the cerebral gangalion of S. maculata is photomicrographically illustrated in Plate No.1, Figs. 1 to 4.

The cerebral ganglia of S.maculata were placed at some distance and they were connected by small band of cerebral commissure. The so called dorsal body was not distinctly observed in this slug.

The histological structure visualised in the HE1,HE2 and MT staining techniques was very much similar. The nerve cells and nerve fibers were stained pink whereas the neurosecretory cells were stained bluish in colour in all the three methods (Plate No.1, Figs. 1 to 4). The nucrosecretory granules were distinctly seen in these cells.

Table 1 : Histological and Histochemical Staining Reactions of Neurosecretory Cells in the Cerebral Ganglion of *Semperula maculata* During Breeding Season

Name of the Technique	Organelles	CELL TYPES							
		TYPE I	TYPE II	TYPE III	TYPE IV	TYPE V	TYPE VI	TYPE VII	TYPE VIII
a) Histological									
1 HE-1	N	B ++++	B ++++	B ++++	B ++++	B ++++	B ++++	B ++++	B ++++
	C	P +++	P +++	P +++	P +++	P +++	P +++	P +++	P +++
2 HE-2	N	B ++++	B ++++	B ++++	B ++++	B ++++	B ++++	B ++++	B ++++
	C	P +++	P +++	P ++	P ++	P ++	P ++	P ++	P ++
3 MT	N	B ++++	B ++++	B++++	B ++++	B ++++	B ++++	B ++++	B ++++
	C	R ++++	R ++++	P ++	P ++	P ++	P ++	P ++	P ++
B) Histochemical									
4 PAS	NG	M ++++	M ++++	M ++++	M ++++	M ++++	M ++++	M ++++	M ++++
5 Feulgen	N	M ++++	M ++++	M ++++	M ++++	M ++++	M ++++	M ++++	M +++
6 AF	NG	P ++++	P ++++	P ++++	P ++++	P ++++	P +++	P +++	P +++
7 AB pH 2.5	NG	B ++++	B ++++	B ++	B +	B +	-	-	-
8 AB pH 1.0	NG	B +++	B +++	B +	B +	-	-	-	-
9 CHP	N	P ++++	P ++++	P ++++	P ++++	P ++++	P ++++	P ++++	P ++++
	NG	BL ++++	BL ++++	BL ++++	BL ++++	BL ++++	BL +++	BL +++	BL ++

N.B. : ++++ = Very intense reaction, +++ = Intense reaction, ++ = Moderate reaction, + = Poor reaction, - = No reaction

Colours : B = Blue, P = Pink, R= Red, BL = Bluish black, M = Magenda.

Abbreviations : N = Nucleus, C = Cytoplasm, NG = Neurosecretory granules, PAS= Periodic acid Shiff, AB= Alcianblue 8 G X 300.

PLATE NO. 1

(Fig. 1 to 6)

(The Neurosecretory Cells in the Cerebral ganglia of S. maculata during breeding season)

Fig. No. 1 Half portion of cerebral ganglia of the slug collected in June & stained with HE Tech. Note. Dorsomedian (DM). Dorsolateral (DL) ventromedian(VM) and Ventrolateral (VL) localities of neurosecretory cells. Nerves (N) and neurons (NC) are seen inbetween these localities. X 540.

Fig.No.2 The same section as seen in Fig.No.1 but stained with MT technique showing four localities (DM.DL.VD.and VL) and nerves (N) and neurons (NC) . X 540.

Fig.No.3 Small portion of cerebral ganglia of slugs collected in July and stained with MT technique showing initiation of neurosecretory activity in neurosecretory cells in four localities (DM.DL.VM and VL) but not in nerves (N) and neurons (NC).X 540.

Fig.No.4 Small portion of cerebral ganglia of slug collected in August and stained with MT technique. Note the maximum staining reactivities of neurosecretory types I(TPI) and Type (TPII) cells. X 900.

Fig.No.5 Small portion of cerebral ganglia of slug collected in September stained with PAS technique. Note Magenda coloured staining of Type I (TPI).Type II(TPII) and Type VIII(TPVIII) cells. Nerves (N) not stained .X 900.

Fig.No.6 Small portion of cerebral ganglia of slug collected in September and stained with AB(pH 2.5) technique. Note decreased staining in Type I(TPI) cells. Axons (A) containing neurosecretory material are clearly seen. Neurons (NC) also stained in this technique. X 900.

(Fig.Nos. 7 and 8)

(Ovotesticular cells of S.,maculata in the breeding season)

Fig.No.7 Small portion of ovotestis of slug collected in early September and stained with HE technique. Note intensely stained germinal epithelium (GE). Sertoli cells(SC). Spermatids (ST). X 900.

Fig.No.8 Small portion of ovotestis late September and stained as in Fig.No.7. but note intensed stained matured sperms (S) besides other cellular elements. X 900.

PLATE NO.2

(Fig. No. 1 to 4)

(The Neurosecretory cells in the cerebral ganglia of S. maculata during aestivation)

Fig.No.1 Small portion of cerebral ganglia of the slug collected in October and stained with cosine showing neurosecretory cells in Dorsomedian(DM), Dorsolateral (DL), Ventromedian(VM) and Ventrolateral (VL) regions. X 540.

Fig.No.2 Small portion of cerebral ganglia of the slug collected in November and stained with AF, showing decreased staining reactivities in neurosecretory cells in four regions (DM,DL,VM and VL). X 540.

Fig.No.3 Small portion of cerebral ganglia of the slug collected in December and stained with PAS. Note reduced size and staining in neurosecretory cells in four regions (DM,DL,VM and VL).X 540.

Fig.No.4 Small portion of cerebral ganglia of slug collected in January, stained with PAS technique showing less number and shrunken size of neurosecretory cells in four regions (DM,DL,VM and VL). Neurons (NC) are intensely stained and nerves (N) not stained at all . X 540.

(Fig.No.5 to 8)

(Ovotesticular cells of S. maculata during aestivation)

Fig.No.5 Few Follicles (F) of ovotestis of slug collected in October and stained with PAS. Note reduced reactivities in germinal epithelium (GE) and sperms (S) . X 900.

Fig.No.6 Few Follicles (F) of ovotestis of slug collected in November and stained with PAS. Note further reduction in staining in germinal epithelium (GE) and sperms (S). Note degenerating ova (O). X 900.

Fig.No.7 Few Follicles (F) of ovotestis of slug collected in December and stained with PAS. Note shrunken size of Ova(O), some degenerating ova (DO), Small spermatids (ST) showing very poor staining. X 900.

Fig.No.8 Few Follicles of ovotestis of slug collected in January. Note only remanants of degenerating and sperms(S). Other cells of the ovotestis have lost their integrity. X 900.

PLATE NO.3

(Fig.Nos 1 to 8)

(The Neurosecretory cells in the cerebral ganglia of C. semirugata during breeding season)

Fig.No.1 Small portion of cerebral ganglia of snail collected in June and stained with HE showing moderate staining of neurosecretory cells at DM.DL.VM and VL regions. Note the septum (S) dividing cerebral ganglia above the oesophagus (O). Nerves (N) are at the centers X 600.

Fig.No.2 Small enlarged portion of the Fig.No.1. Note type I (TPI), Type III(TP III) and Type V(TPV) neurosecretory cells. X 2000.

Fig.No.3 Small portion of cerebral ganglia of snail collected in July and stained in HE, showing increased size and staining intensities of neurosecretory cells of four regions (DM.DL.VM and VL) above the oesophagus (O). Neurons (NC) are intensely stained and nerves (N) are not stained . X 600.

Fig.No.4 Other portion of the cerebral ganglia of the Fig.No.3. Note Dorsal body (DB) with distinct neurosecretory cells. Cerebral ganglia, is divided by connective tissue strand (CO) above the oesophaglus (O). X 600.

Fig.No.5 Small portion of cerebral ganglia of snail collected in August and stained in MT technique. Note Type I (TP I), Type II (TP II) and Type III (TP III) cells with red nuclei and bluish neurosecretory material. X 600.

Fig.No.6 Small portion of cerebral ganglia as in Fig.No.5 but stained with CHP technique. Note intensely stained neurosecretory cells (TP II, TP IV). X 600.

Fig.No.7 Small portion of cerebral ganglia of snail collected in September stained with PAS, showing intensely staining neurosecretory cell types, I,III and VII (TP-I,TP-III and TP-VII). X 2000.

Fig.No.8 Small portion of cerebral ganglia as in Fig.No.7 but stained with AF technique. Note AF positive neurosecretory cell types (TP-I, TP-II and TP-III). Nucleus (N) is not stained. X 2000.

PLATE NO.4

(Figs. No.1 to 8)

(Ovotesticular cells of C. semirugata during breeding season)

Fig.No.1 Few Follicles (F) of ovotestis of snail collected in June stained with HE. Note germinal epithelium (GE) and ova (O). X 600.

Fig.No.2 Small portion of follicle of Fig.No.1 showing intensely stained ovum (O). Germinal epithelium (GE) and Sperms (S). X 2000.

Fig.No.3 Few Follicles (F) of ovotestis of snail collected in July and stained with MT technique. Note increased staining in germinal epithelium (GE), ova and Sperms (S). X 1500.

Fig.No.4 Few Follicles of ovotestis as in case of Fig.No.3. Note intensely stained nurse cells (NC), Spermatids (ST), Ova(O). X 1500.

Fig.No.5 Few Follicles of ovotestis of snail collected in August and stained with MT technique. Note cortical granules (C) at the periphery of Ovum (O). Germinal epithelium (GE) and Spermatids (ST) intensely stained. X 1500.

Fig.No.6 A single Follicle (F) showing Ovum (O), nurse cell (NC) of Fig.No.5 but stained with HE. X 1500.

Fig.No.7 Some portion of single Follicle of snail collected in September stained with PAS, showing Sperms (S) germinal epithelium (GE) intensely stained. X 2000.

Fig.No.8 Few Follicles (F) of the Fig.No.7 showing Ovum (O), Sperms (S) and germinal epithelium (GE) intensely stained. X 1500.

PLATE NO.5

(Fig.Nos. 1 to 8)

(The Neurosecretory cells in cerebral ganglia of C. semirugata during aestivation)

Fig.No.1 Small portion of cerebral ganglia of snail collected in October and stained with HE, showing DM,DL, VM and VL regions containing neurosecretory cells. Nerves (N) are not stained .X 540.

Fig.No.2 Few neurosecretory cells of type I(TPI) with their axons (A) of the Fig.No.1 and stained with PAS. X 2000.

Fig.No.3 Small portion of cerebral ganglia of snail collected in December and stained with PAS. Note neurosecretory cells in Dorsal body (DB) and in four regions DM,DL,VM and VL. Nerves (N) are not stained. X 560.

Fig.No.4 Small portion of Fig.No.3 enlarged showing Type I, II and III cells (TP I,TP II, TP III).X 2000.

Fig.No.5 Small portion of cerebral ganglia of snail collected in February and stained with PAS. Note decreased size and staining of neurosecretory cells. X 560.

Fig.No.6 Enlarged neurosecretory cell of snail collected in February and stained with AF technique. Note very few neurosecretory granules (NG) in these cells and axons (A). X 2000.

Fig.No.7 Small portion of cerebral ganglia stained with AB pH 2.5 of snail collected in April, showing very reduced size and number of neurosecretory cells in each region (DM,DL,VM and VL). X 560.

Fig.No.8 Few cells of the Fig.No.7 enlarged showing empty or few neurosecretory granules (NG) in Type I (TPI), Type II(TP II) and Type III (TP III) cells. X 2000.

PLATE NO.6

Fig.Nos 1 to 4

(Ovotesticular cells of C. semirugata during aestivation)

Fig.No.1 Few Follicles (F) of ovotestis stained with PAS of snails collected in October, showing reduced staining reactivities in germinal epithelium (GE) and Ova(O). X 600.

Fig.No.2 Few Follicles (F) of ovotestis stained with HE of snails collected in November, showing shrunken germinal epithelium (GE) and other cells also. X 600.

Fig.No.3 Few Follicles (F) of ovotestis stained with HE of snails collected in December. Note reduced size of Ova (O) and few spermatids only. X 540.

Fig.No.4 Few Follicles (F) of ovotestis stained with HE of snails collected in January without any sperms. smaller nurse cells (NC) and degenerating Ova (O). X 540.

Fig.Nos. 5 to 8

(Neurosecretory cells in optic tentacles during breeding season and aestivation)

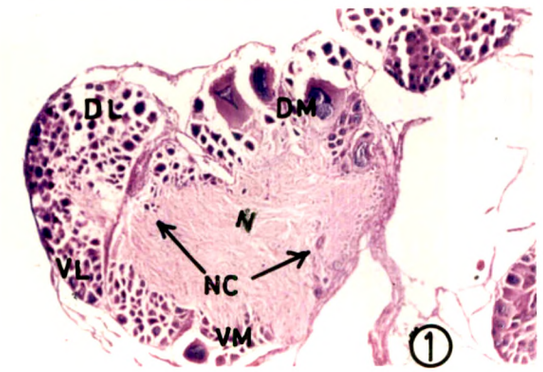
Fig.No.5 L.S. of optic tentacles stained with MT of snail collected in July showing neurosecretory collar cells (NC). connective tissue (CT) and muscles (M). X 600.

Fig.No.6 L.S. of optic tentacles of snail collected in August, stained with MT showing intense staining in neurosecretory cells (NC). X 600.

Fig.No.7 L.S. of optic tentacles of snail collected in February, stained with PAS, showing reduced reactivities of neurosecretory cells (NC). Epithelial cells (EP) and muscles (M) are moderately stained.X 600.

Fig.No.8 L.S. of optic tentacles of snail collected in April showing shrunken size of epithelium (EP) and neurosecretory cells (NC). X 600.

Semperula maculata



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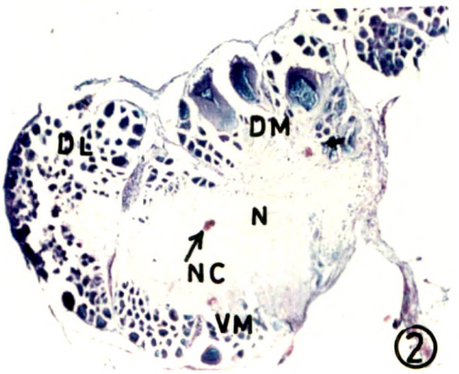

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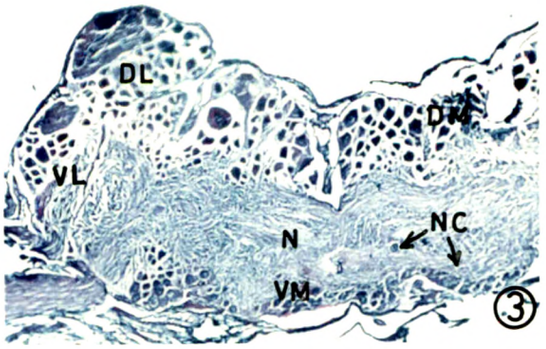

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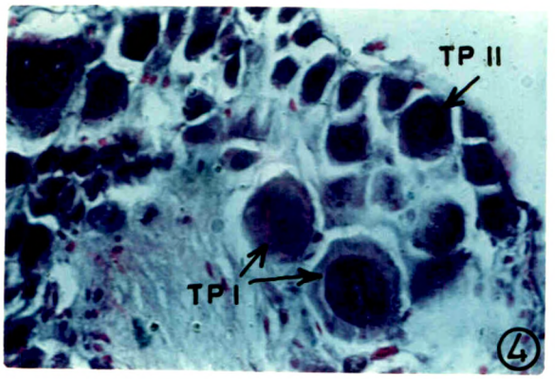

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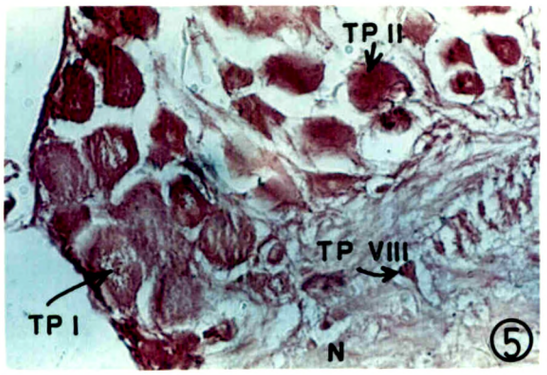

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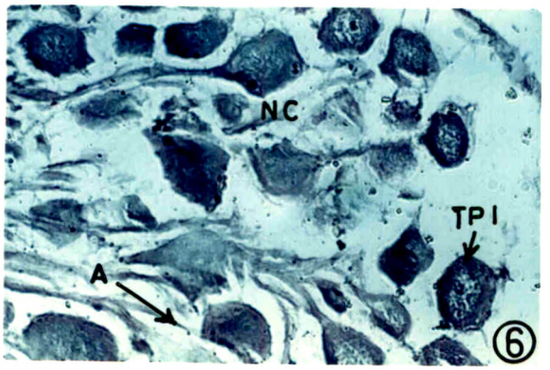

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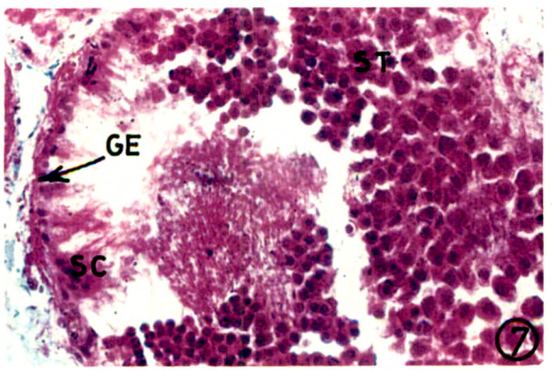

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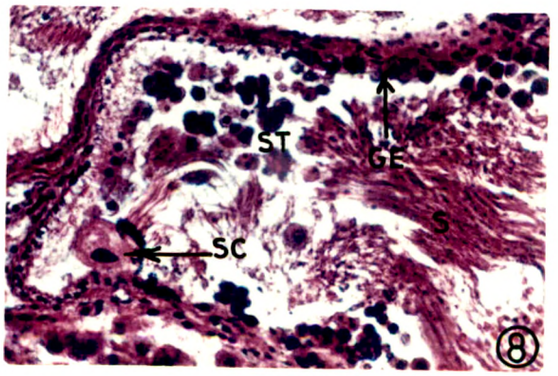

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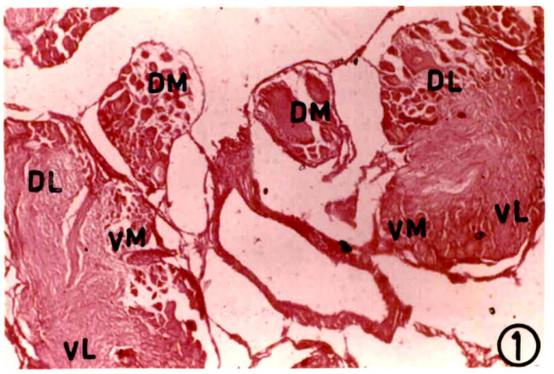
PLATE No.1

Semperula maculata



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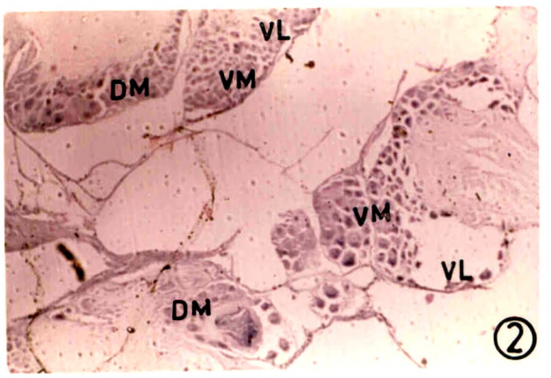

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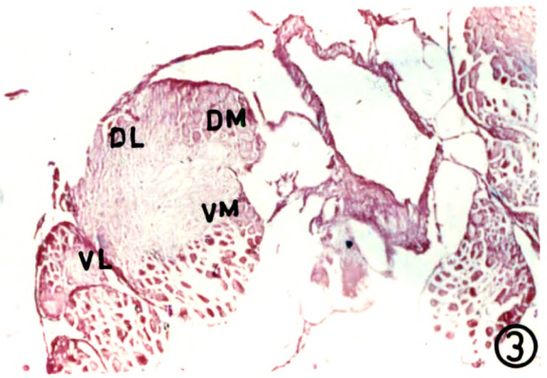

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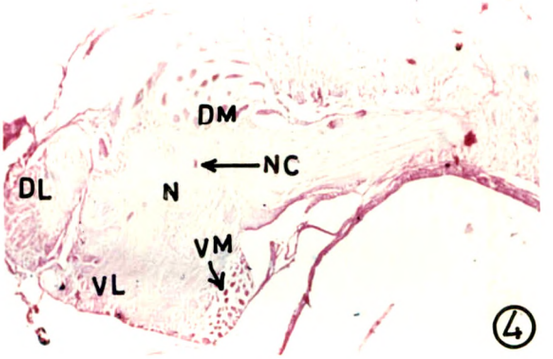

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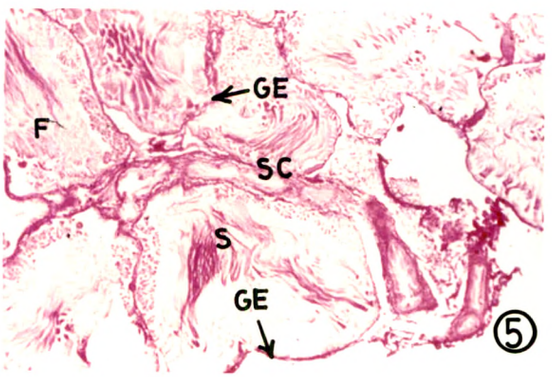

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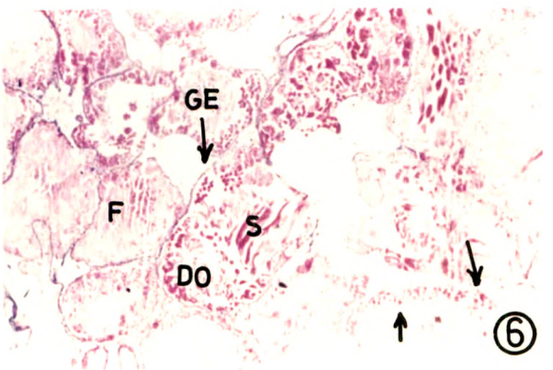

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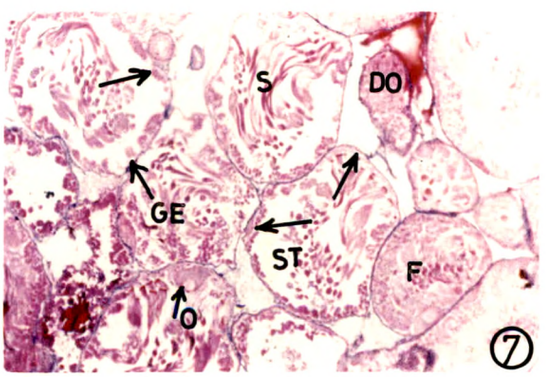

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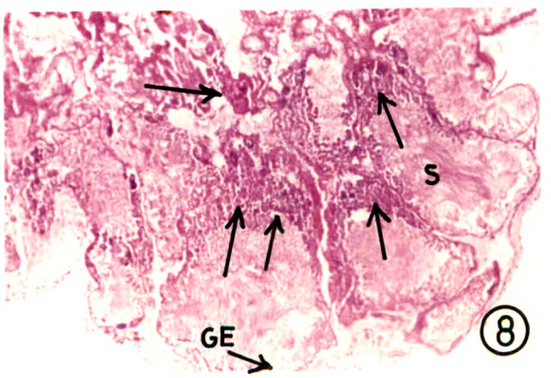

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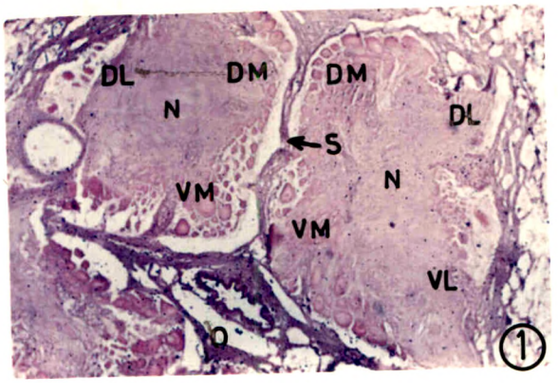
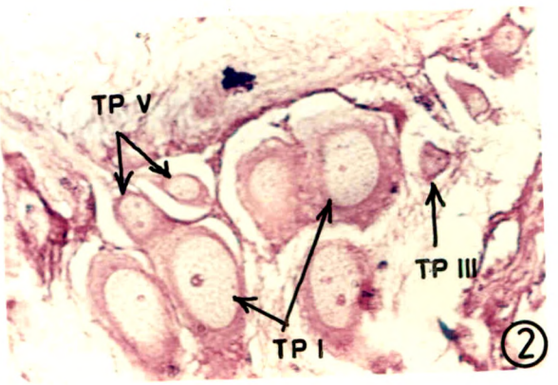
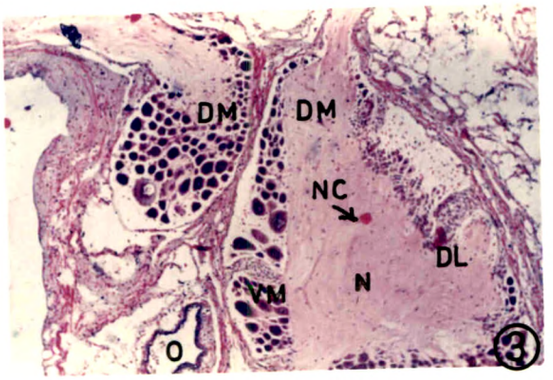
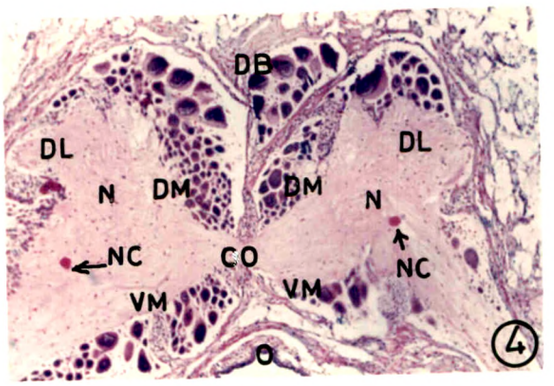
PLATE No. 2

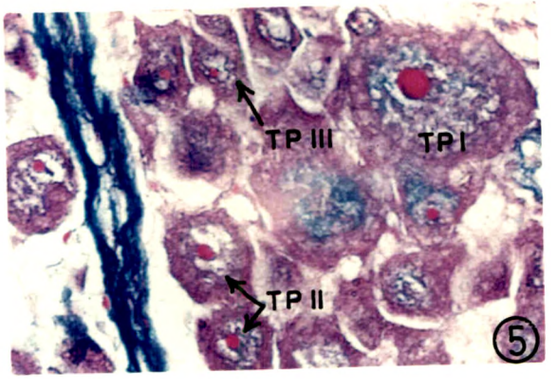
Cryptozona semirugata

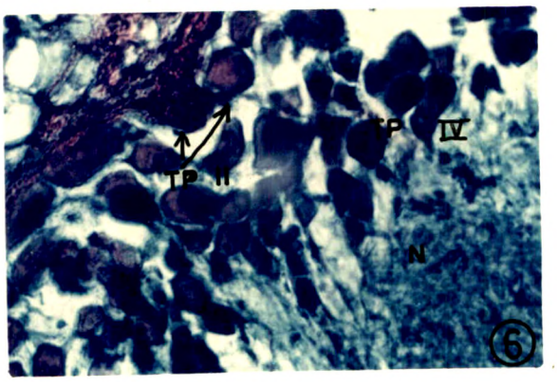


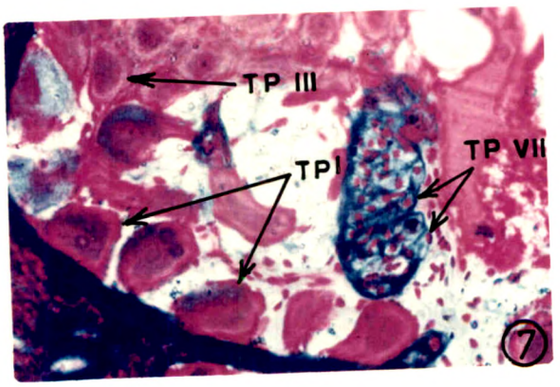












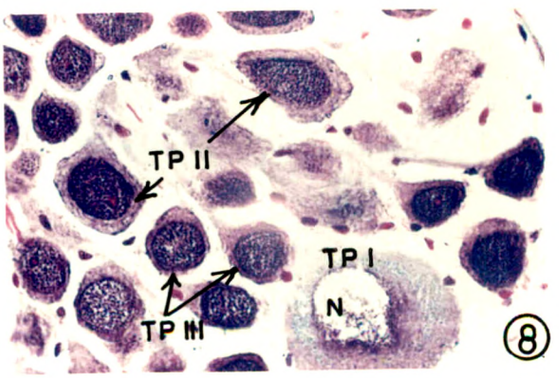
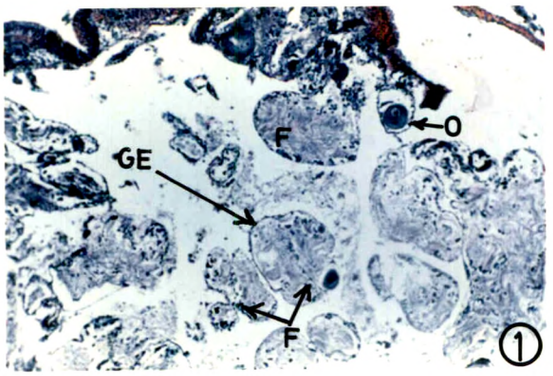


PLATE No. 3

Cryptozona semirugata



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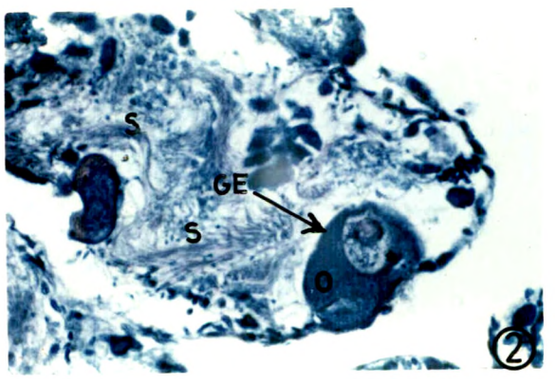

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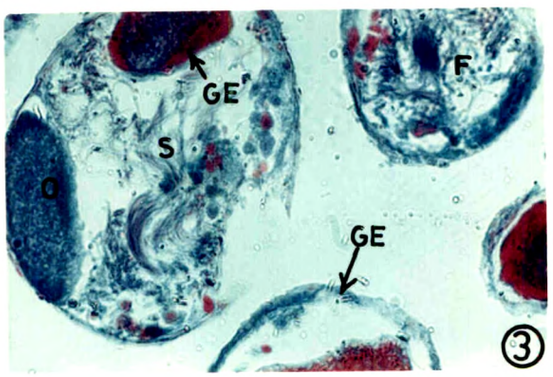

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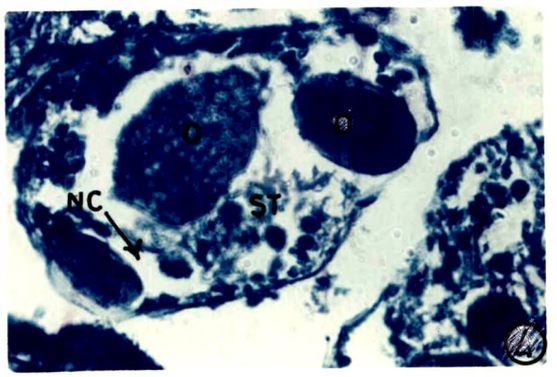

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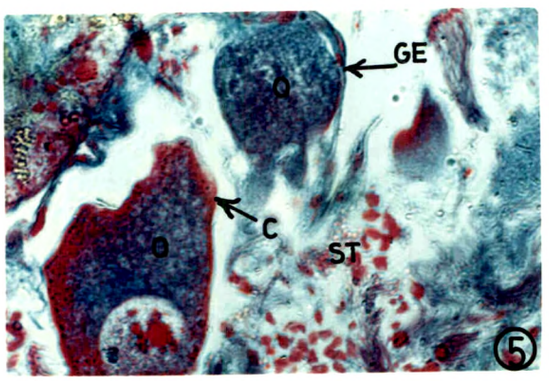

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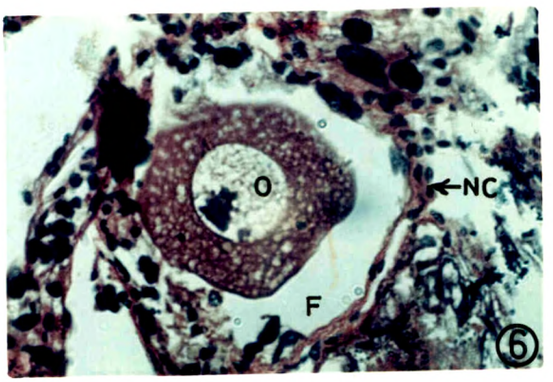

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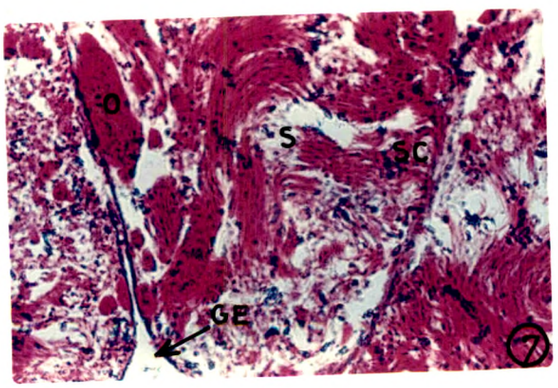

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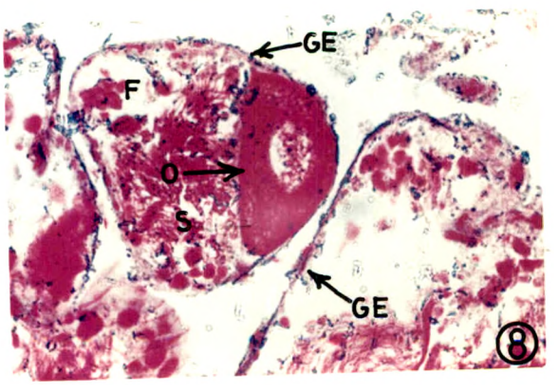


⑧

PLATE No. 4

Cryptozona semirugata

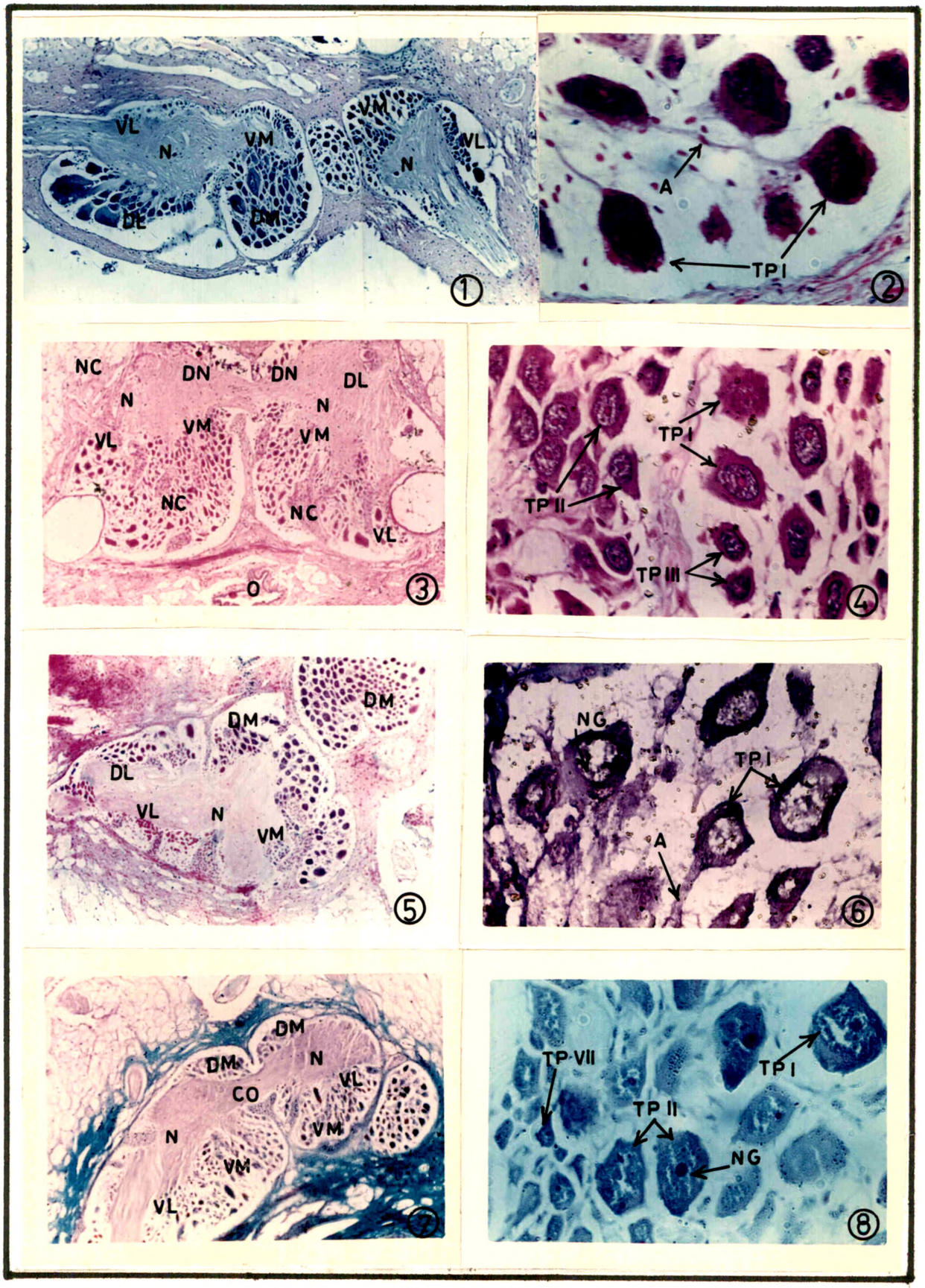
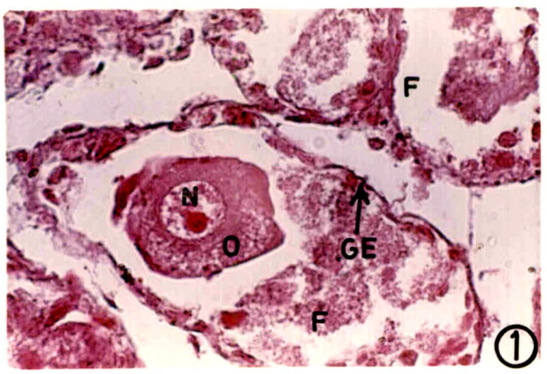
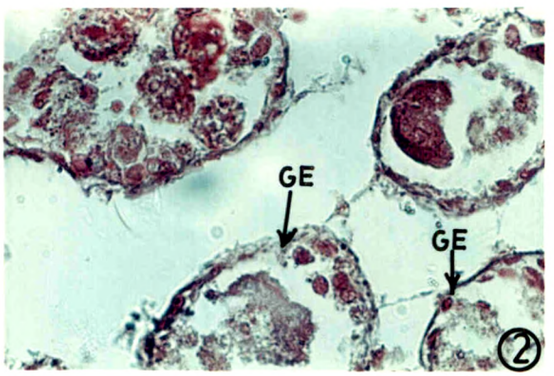
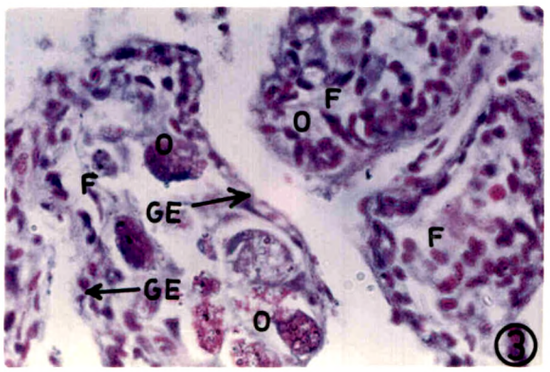


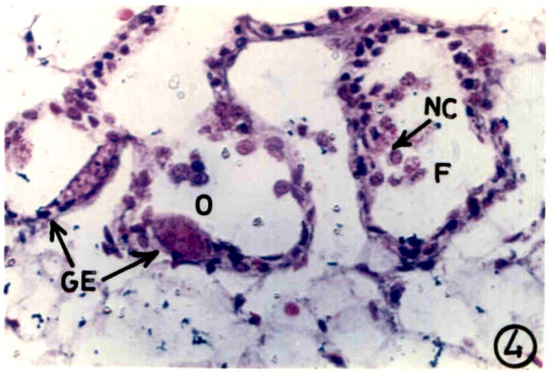
PLATE No. 5

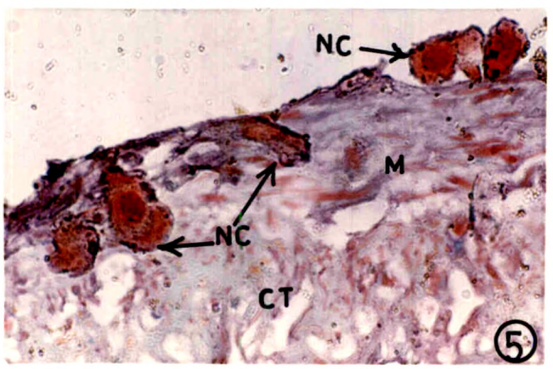
Cryptozona semirugata

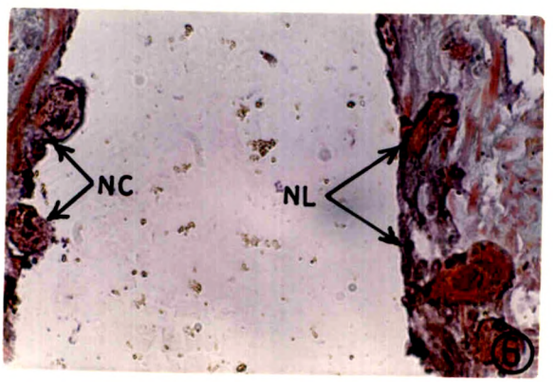


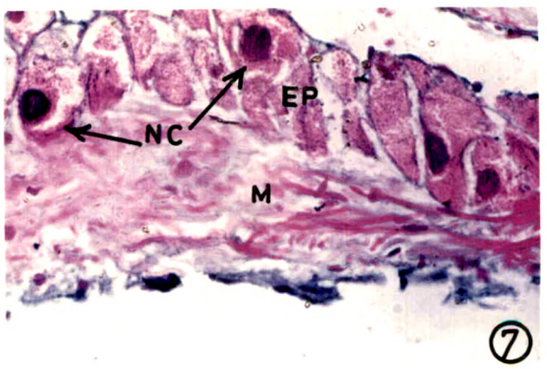












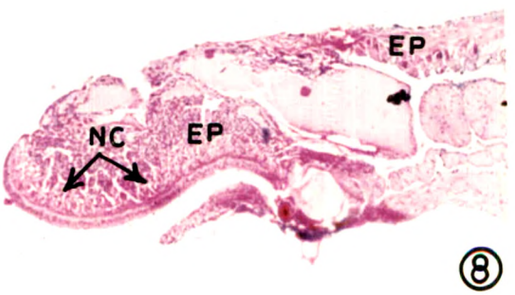


PLATE No. 6

The neurosecretory cells were not uniformly distributed all over the cerebral ganglion but they were grouped at certain regions of the cerebral ganglion. On the basis of the localization of the neurosecretory cells, four regions could be distinctly marked i.e. Dorsomedian region, Dorsolateral region, Ventromedian region and Ventrolateral region, which were designated as DM,DL,VM and VL, respectively (Plate No.1, Figs. 1 and 2).

On the basis of the size and staining intensities of the neurosecretory cells localized in the above mentioned regions they could be distinguished into eight types. The largest cells were designated as Type I cells and the smallest sized cells were designated as Type VIII cells. The remaining cells of the same size but in the decreasing order were designated as Type II, Type III, Type IV, Type V,Type VI, and Type VII cells. Type I cells are like α cells which have been described in other molluscan species and remaining types can be considered as β cells.

Another important point of academic interest was that the number of each type of cells was not uniform in the above mentioned four regions (DM, DL,VM and VL). This could be seen by observing the relative number of the various types of cells in the sections of the cerebral ganglion. It was also noticed that the relative number of the eight types of cells varied during the entire period of breeding season. Such differences in the number of cells made it incumbent on us to count exactly the number of cells belonging to a particularly type in the sections of 20 slides per month of 10 different specimens and take the average

for a section for the particular period (early, mid or late) of the breeding season. The variations in the number of the eight types of neurosecretory cells during three periods of the breeding season have been presented in Table No.2.

At a comparative level, the number ofType VII and Type VIII cells, was the heighest in all the four regions of the cerebral ganglion. The number of remaining cells types decrease with the decreasing order of the cell types.

Among the four regions, the ventromedian region contained the maximum number of cells, ventrolaterial and dorsolateral regions contained moderate number of cells whereas the dorsomedian region contained the lowest number of cells.

The regional distribution of relative number of the neurosecretory cells was not consistently the same in all the three periods of the breeding season. The number of neurosecretory cells in the early breeding period was minimum showing 337 cells . The number of these cells was maximum, 466 in the mid breeding period and it was decreased to 446 in the late breeding period.

B) HISTOCHEMICAL OBSERVATIONS ON CEREBRAL GANGLION DURING BREEDING SEASON :

The histochemical data on some important histochemical staining techniques employed in the present investigation of cerebral ganglion during breeding season are recorded in Table No.1. The chemical nature and concentration of the contents of the neurosecretory cells were detected by

Table -2 : The Numerical Changes in Neurosecretory Cells in the Various Regions of Cerebral Ganglion of Semperula maculata During Breeding Season

Phase of the Breeding season	Region of cerebral ganglion	C E L L T Y P E S							
		TYPE I	TYPE II	TYPE III	TYPE IV	TYPE V	TYPE VI	TYPE VII	TYPE VIII
1 Rarly Breeding Season	DM	2	4	4	4	4	4	6	4
	DL	1	1	2	8	6	10	14	20
	VM	1	1	1	14	24	12	40	30
	VL	2	2	4	8	12	10	42	40
2 Mid Breeding Season	DM	2	4	4	4	6	6	6	8
	DL	1	1	2	10	10	16	18	30
	VM	1	1	1	18	32	15	50	40
	VL	2	4	8	10	16	20	60	60
3 Late Breeding Season	DM	2	4	4	4	6	6	6	6
	DL	1	1	2	10	8	14	16	28
	VM	1	1	1	17	30	14	46	40
	VL	2	4	8	10	16	18	60	60

visually estimated intensity and shade with four plus (++++) representing the strongest activity. The histochemical observations requiring further description and consideration are presented hereafter. The histochemical distribution of neurosecretory granules in the cells of the cerebral ganglion of S. maculata is photomicrographically illustrated in Plat No.1, Figs 5 and 6.

The neurosecretory cells stained purplish-red in PAS staining technique (Plate No.1, Fig.5). indicating the presence of glycogen or /and polysaccharides in them. The polysaccharide contents showed alcianophilia at pH 2.5 indicating its acidic nature. The Deep-purple staining in AF method and blue colour in CHP method (Plate No.1, Fig.6). indicate the presence of neurosecretory material in these cells. Axons of cells carrying these neurosecretory material is clearly seen in the sections (Plate No.1, Fig.5 and 6).

The concentration of neurosecretory products was not same in all the cell types nor it remained constant in the particular cell type throughout the breeding season. Its concentration was minimum in the early breeding period which attained its heighest peak in the mid-breeding period. It was progressively declined in the late breeding period. Such variations could be visualised by observing the sections stained in various histochemical methods.

C) *HISTOLOGICAL OBSERVATIONS ON OVOTESTIS DURING BREEDING SEASON* :

The histological data on the histological staining techniques employed in the present investigation of ovotestis during breeding season are recorded in

Table No.3. The histological distribution of various ovotesticular cellular elements in the ovotestis of S. maculata is photomicrographically illustrated in Plate No.1, Figs. 7 and 8.

Histologically, germinal epithelial cells, nutritive cells, Stertoli cells, spermatids, sperms and ova could be distinguished by the histological staining techniques (HE1, HE2, MT and Feulgen). The number and staining intensity of these cells varied in every cellular element during different phases of breeding season. The number and staining intensities were maximum in the mid breeding period and moderate in the early and late breeding periods.

D) ***HISTOCHEMICAL OBSERVATIONS ON OVOTESTIS DURING BREEDING SEASON*** :

The histochemical data of some important staining techniques employed in the present investigation of ovotestis during breeding season are recorded in Table No.3 . The histochemical distribution and variations of different chemical constituents in ovotesticular cells of S. maculata are photomicrographically illustrated in Plate No.1, Figs. 7 and 8.

Observations on various histochemical methods indicted the presence of glycogen and neutral mucosubstances in the germinal epithelial cells. The nutritive cells contained higher concentration of glycogen and less concentration of neutral mucosubstances. The Sertoli cells also contained higher concentration of glycogen. The spermatids contained neutral and acidic mucopolysacharides. The matured sperms contained glycogen in their tails and hyaluronic acid in

Table - 3 : Histological and Histochemical Staining Reactions of Ovotesticular Cells of Semperula maculata During Breeding Season

Name of the Technique	Phase of the Breeding Season	Ovotesticular Cell Types					
		Germinal Epithelial cell	Nutritive cell	Serttoli cell	Spermatid	Sperm	Ovum
1 HE	Early Breeding	+++	++	++	+	++	++
	Mid Breeding	++	+++	+++	++	+++	++++
	Late Breeding	++	++	++	+++	++	+++
2 MT	Early Breeding	+++	++	++	+	++	++
	Mid Breeding	++	+++	+++	++	+++	++++
	Late Breeding	++	++	++	+++	++	+++
3 PAS	Early Breeding	+++	++	++	++	++	++
	Mid Breeding	+	++++	++++	+++	+++	++++
	Late Breeding	++	+++	+++	+++	++	+++
4 AF	Early Breeding	-	-	-	-	-	-
	Mid Breeding	-	-	-	-	-	-
	Late Breeding	-	-	-	-	-	-
5 AB pH 2.5	Early Breeding	+	+	+	+	++	++
	Mid Breeding	+	+	+	+	+++	++++
	Late Breeding	+	+	+	+	++	+++
6 AB pH1.0	Early Breeding	-	-	-	-	-	-
	Mid Breeding	-	-	-	-	-	-
	Late Breeding	-	-	-	-	-	-

N.B. : ++++ = Very intense reaction, +++ = Intense reaction, ++ = Moderate reaction, + = poor reaction,, - = No reaction,

Abbreviations : PAS = Periodic Acid Schiff, AB = Alcian blue 8G X 300.

their acrosomal part of the head regions. The ovum contained glycogen and sialic acid at their contex zone. These chemical constituents are involved in the formation of cortical granules as suggested by Nanaware (1974).

The chemical components localized in all the ovotesticular cells were not constant throughout the breeding season. Their concentrations started increasing in the early breeding period and reached their maximum levels during mid-breeding period and attained the decreased levels in the late-breeding period.

Secondly, the concentration of one chemical constituent was decreased in germinal epithelial cells when the concentration of that constituent was increased in nutritive or sertoli cells. The concentration of glycogen was decreased in sertoli cells simultaneously it was increased in sperm tails.

The increase and the decrease in the constituents in the ovotesticular cells coincided with the increase and decrease in the number and staining intensities of neurosecretory cells in the cerebral ganglion of this slug.

E) HISTOLOGICAL OBSERVATIONS ON CEREBRAL GANGLION DURING AESTIVATION PERIOD

The histological data on histological staining techniques employed in the present investigation of cerebral ganglion during aestivation period are recorded in Table No.4. The important histological observations are presented hereafter. The histological distribution of various neurosecretory cells and their variations

Table - 4 : Histological and Histochemical Staining Reactions of Neurosecretory Cells in the Cerebral Ganglion of Semperula maculata During Aestivation Period

Name of the technique	Organelles	C E L L T Y P E S							
		TYPE I	TYPE II	TYPE III	TYPE IV	TYPE V	TYPE VI	TYPE VII	TYPE VIII
A) Histological									
1 HE 1	Nucleus	B +++	B ++	B ++	B +	B +	B +	-	-
	Cytoplasm	P +++	P +++	P ++	P ++	P ++	P +	P +	P +
2 HE 2	Nucleus	B +++	B ++	B ++	B +	B +	B +	-	-
	Cytoplasm	P +++	P +++	P ++	P ++	P ++	P +	P +	P +
3 MT	Nucleus	B +++	B +++	B ++	B +	B +	B +	-	-
	Cytoplasm	R +++	R +++	R ++	R ++	R ++	R +	R +	R +
B) Histochemical									
4 PAS	NG	M +++	M ++	M ++	M +	M +	M +	-	-
5 Feulgen	N	M +++	M ++	M ++	M +	M +	M +	-	-
6 AF	NG	P +++	P ++	P ++	P +	P +	P +	-	-
7 AB pH 2.5	NG	B ++	B +	B +	-	-	-	-	-
8 AB pH 1.0	NG	B +	-	-	-	-	-	-	-
9 CHP	N	P +++	P ++	P ++	P +	P +	P +	-	-
	NG	BL +++	BL ++	BL ++	BL +	BL +	BL +	-	-

N.B. : ++++ = Very intense reaction, +++ = Intense reaction, ++ = Moderate reaction, + = Poor reaction, - = No reaction,

Colours : B= Blue, P= Pink, R= Red, BL = Bluish black, M = Magenda.

Abbreviations : N = Nucleus, C= Cytoplasm, NG = Neurosecretary granules, PAS= Periodic acid Shiff, AB = Alcian blue 8G X 300.

in the number and staining in the cerebral ganglion of S.maculata is photomicrographically illustrated in Plate No.2, Figs. 1 and 2.

In general , the staining intensities of various cell types (I to VIII) were decreased considerably. So also the number of each type of neurosecretory cells was reduced to about half as compared to the number in the breeding season and some of the cell types were not observed.The size of all the cells were reduced and they were shrunken. The number of neurosecretory cells was considerably reduced from the four regions (DM,DL,VM and VL) of the cerebral ganglion. The variations in the number of the eight types of neurosecretory cells during aestivation have been represented in Table No.5.

At a comparative level the number of cells was more or less similar in the beginning of the aestivation to that of the number observed in late breeding season , which progressively decreased and attained a minimum level in the mid-aestivation period. Thereafter just before the onset of the breeding season, the number of neurosecretory cells again started increasing and reached a level to that of early breeding season.

F) HISTOCHEMICAL OBSERVATIONS ON CEREBRAL GANGLION DURING AESTIVATION PERIOD :

The histochemical data on various histochemical staining techniques employed in the present investigation of cerebral ganglion during aestivation period are recorded in Table No.4. The important histochemical observations are presented hereafter. The distribution of neurosecretory products, number of the

neurosecretory cells and their staining reactivities in the cerebral ganglion of S. maculata during aestivation are photomicrographically illustrated in Plate No.2, Figs. 3 and 4. The numerical changes have been recorded in Table 5.

The histochemical observations indicated over all increase in the staining intensities of neurosecretory cells. The polysaccharide contents (both glycogen and acid mucopolysaccharides) of these cells were reduced considerably and very faint magenda colour was observed in PAS staining procedure. The neurosecretory granules also reacted poorly towards AF and CHP staining techniques.

The number of neurosecretory cells in each region (DM,DL,VM and VL) was almost reduced to half and the size of these cells was minimised. The number of neurosecretory cells varied in various phases of the aestivation period, which has been already recorded in the histological observations.

G) HISTOLOGICAL OBSERVATIONS ON OVOTESTISC DURING AESTIVATION PERIOD

The histological data on histological staining techniques employed in the present investigation of ovotestis during aestivation are recorded in Table No.6. The important histological observations are presented hereafter.

Comparatively, the staining intensities of each type of ovotesticular cells and their numbers were reduced considerably. Their shapes were abnormal and thet wereshrunken in size . The germinal epithelial cells were stained moderately, nutritive cells were poorly stained, Sertoli cells were not visible in

Table - 5 : The Numerical Changes in Neurosecretory Cells in the Various Regions of Cerebral Ganglion of Semperula maculata During Aestivation

Phase in the Aestivation	Region of Cerebral Ganglion	CELL TYPES							
		TYPE I	TYPE II	TYPE III	TYPE IV	TYPE V	TYPE VI	TYPE VII	TYPE VIII
1 Early Aestivattion	DM	2	2	2	4	4	6	6	8
	DL	1	1	2	8	8	12	16	24
	VM	1	1	2	16	28	14	44	34
	VL	2	2	6	8	14	16	50	50
2 Mid Aestivation	DM	1	1	2	2	2	2	6	4
	DL	1	1	2	4	4	8	10	16
	VM	1	1	2	10	20	10	36	25
	VL	1	1	4	4	10	8	40	32
3 Late Aestivation	DM	1	1	2	2	4	4	6	6
	DL	1	1	2	6	6	10	12	20
	VM	1	1	2	12	24	12	40	30
	VL	1	1	2	6	12	10	44	34

the histological staining preparations. The number of spermatids was increased and concomittantly the number of sperms was reduced. The tail length of sperms was reduced. The ova were degenerated.

Degenerative and reductive changes in the cells attached with the male sex cells and female sex cells were started in the early aestivation period and reacted their maximum levels in mid-aestivation which were retained throughout the active aestivation period. This situation was changed only in the last aestivation phase and normal size, shape and number of these cells were attained at the beginning of breeding season.

H) HISTOCHEMICAL OBSERVATIONS ON OVOTESTIS DURING AESTIVATION PERIOD :

The histochemical data on various histochemical staining procedures employed in the present investigation of ovotestis during aestivtion period are recorded in Table No.6.

The important histochemical observations are presented hereafter. The staining reactivities shown by various cells in the ovotestis of S. maculata during aestivation are photomicrographically illustrated in Plate No.2, Figs. 5 to 8.

At a comparative level the staining reactivities shown by all the ovotesticular cells were very much reduced. PAS staining was seen only in the germinal epithelial cells, sperm heads and tails only (Plate No.2, Fig.5), in the early aestivation period. During active aestivation period PAS reactivity was shown by disintegrating ova and by remanants of sperms (Plate No.2, Fig. 6 and

Table - 6 : Histological and Histochemical Staining Reactions of Ovotesticular Cells of Semperula maculata During Aestivation

Name of the technique	Phase of the aestivation	OVOTESTICULAR CELL TYPES					
		Germinal epithelial cell	Nutritive cell	Sertoli cell	Spermatid	Sperm	Ovum
1 HE	Early aestivation	++	+	+	+	++	+
	Mid aestivation	+	-	-	+	+	-
	Late aestivation	++	+	+	+	++	+
2 MT	Early aestivation	++	+	+	+	++	+
	Mid aestivation	+	-	-	+	+	-
	Late aestivation	++	+	+	+	++	+
3 PAS	Early aestivation	++		+	+	++	+
	Mid aestivation	+	-	-	+	+	-
	Late aestivation	++	+	+	+	++	+
4 AF	Early aestivation	-	-	-	-	-	-
	Mid aestivation	-	-	-	-	-	-
	Late aestivation	-	-	-	-	-	-
5 AB pH 2.5	Early aestivation	-	-	-	-	+	+
	Mid aestivation	-	-	-	-	-	-
	Late aestivation	-	-	-	-	+	+
6 AB pH 1.0	Early aestivation	-	-	-	-	-	-
	Mid aestivation	-	-	-	-	-	-
	Late aestivation	-	-	-	-	-	-

N.B. : ++++ = Very intense reaction, +++ = Intense reaction, ++ = Moderate reaction, + = Poor reaction, - = No reaction,

Abbreviations : PAS = Periodic Acid Schiff, AB = Alcian Blue 8G X 300.

7). But again the increase in the PAS reactivity was noted in the late aestivation period due to termination of inactive life phase of the slug (Plate No.2, Fig.8).

2) CRYPTOZONA SEMIRUGATA :

A) HISTOLOGICAL OBSERVATIONS ON CEREBRAL GANGLION DURING BREEDING SEASON :

The histological data of cerebral ganglion of this snail during breeding season are recorded in Table No.7, according to the visually estimated intensity and shade with four plus (++++) representing the strongest activity. The important histological observations are presented hereafter. The Histological distribution of neurosecretory cells in the cerebral ganglion of C. semirugata is photomicrographically illustrated in Plate No.3, Figs. 1 ,3 and 4.

The lobes of cerebral ganglia of C. semirugata were located very close to each other and were separated by dorsal and ventral furrows. They were connected to each other by small strip of commissure. Thus perfect bilateral symmetry could be observed in the section (Plate No.3, Figs. 1,3 and 4). The dorsal bodies were clearly seen and the neurosecretory cells were filled in them.

The neurosecretory cells were distinctly seen in the cerebral ganglion in all the histological staining procedures. They contained neurosecretory granules of various sizes in them. These cells located at the peripheral regions of the cerebral ganglia. They were in groups. Like S. maculata, based on the localization of these neurosecretory cells four regions of the cerebral ganglia could be distinctly marked i.e. Dorsomedian region, Dorsolateral region,

Table 7 : Histological and Histochemical Staining Reactions of Neurosecretory cells in the Cerebral Ganglion of Cryptozona semirugata During Breeding Season

Name of the technique	Organelles	CELL TYPES						
		TYPE I	TYPE II	TYPE III	TYPE IV	TYPE V	TYPE VI	TYPE VII
A) Histological								
1 HE 1	N	B ++++	B ++++	B +++	B +++	B ++	B ++	B +
	C	P ++++	P ++++	P ++++	P ++++	P ++++	P ++++	P +++
2 HE 2	N	B ++++	B ++++	B +++	B +++	B ++	B ++	B +
	C	P ++++	P ++++	P ++++	P ++++	P ++++	P ++++	P +++
3 MT	N	R ++++	R ++++	R +++	R +++	R ++	R ++	R +
	C	B ++++	B ++++	B ++++	B ++++	B ++++	B ++++	B +++
B) Histochemical								
4 PAS	NG	M +++	M +++	M +++	M ++	M ++	M ++	M ++
5 Feulgen	N	M +++	M +++	M +++	M ++	M ++	M ++	M +
6 AF	NG	P +++	P +++	P +++	P ++	P ++	P ++	P +
7 AB pH 2.5	NG	B ++	B +	B +	B +	-	-	-
8 AB pH 1.0	NG	B +	-	-	-	-	-	-
9 CHP	N	P ++++	P ++++	P +++	P ++	P +	P +	-
	NG	BL +++	BL ++	BL +	BL +	BL +	BL +	BL +

N.B. : ++++ = Very intense reaction, +++ = Intense reaction, ++ = Moderate reaction, + = Poor reaction, - = No reaction

Colours = B = Blue, P=Pink, R = Red, BL = Bluish black.

Abbbreviations : N= Nucleus, C= Cytoplasm, NG= Neurosecretory Granules PAS=Periodic Acid Schiff, AB=Alcian Blue 8GX300.

Ventomedian region and Ventrolateral region. The number of neurosecretory cells varied in each of these regions.

On the basis of the size and staining intensities of the neurosecretory cells cculd be classified into seven types. Type I (or α cells) were the largest cells and remaining small sized cells (or β cells) could be grouped into Type II, Type III, Type IV, Type V, Type VI and Type VII cells in the decreasing order of size. Dorsal bodies contained only one (α type type) of cells whereas cerebral ganglia contained the seven types of cells in them .

The histological structure of all the seven types of neurosecretory cells HE1,HE2andMT staining procedures was very much similar. The nerve cells and nerve fibers were stained pink whereas all the neurosecretory cells were stained blush in colour in all the three methods.

At a comparative level the sizes all the types of cells in C.semiragata were larger than their corresponding types in S.maculata. Intensities of staining were also more than those in S.maculata. The neurosecretory granules were large and they were distinctly observed.

The four regions contained variable number of different cell types. This could be observed by noting the relative number of the various types of the cells in the sections of cerebral ganglia. The relative number of cells was not only varied in different regions of the cerebral ganglia but also in various phases of the breeding season. Such differences in the number of cells made it incumbent on us to count exactly the number of cells belonging to a particular type in the

sections of 20 slides per month of 10 different specimens and take the average for a section for the particular phase (early, mid and late) of the breeding season. Such variations in the number of the seven types of neurosecretory cells during three phases of the breeding season have been presented in Table No.8.

B) HISTOCHEMICAL OBSERVATIONS ON CEREBRAL GANGLION DURING BREEDING SEASON :

The histochemical data on some important histochemical staining techniques employed in the present investigation of cerebral ganglia during breeding season are recorded in Table No.7. The neurosecretory products of all the cell types listed in the histological observations were seen in the form of granules (neurosecretory granules). The concentration and intensity of neurosecretory cells depended on the number and staining capabilities of these granules. The concentration and shade of the contents of the cells were detected by usually estimated intensity with four plus (++++) representing the strongest activity. The histochemical distribution of the cells of the cerebral ganglia of C. semirugata is photomicrographically illustrated in Plate No.3, Figs.2 ,5 to 8.

The cells stained pink in PAS staining technique (Plate No.3, Fig.2) indicating the presence of glycogen or/and polysaccharides in them. The polysaccharide contents showed poor alcianophilia at pH 2.5. But the cells were stained deep-purple in AF and bluish in colour in CHP method (Plate No.3, Fig.6). All the cell types did not show the same intensity of colour nor they

showed uniform single colour in sequential staining procedures (Plate No.3, Fig.5,,7 and 8). The observations on all these staining procedures indicted the presence of glycogen and some acidic muscosubstances along with the neurosecretory products.The numerical changes have been recorded in Table 8.

The concentration of neurosecretory materials varied in different cell types during various phases of the breeding season. The concentration of most of the cell types was low at the beginning of early breeding period .The concentrations reached the heighest during the mid-breeding period which declined in the late breeding phase.

C) HISTOLOGICAL OBSERVATIONS ON OVOTESTIS DURING BREEDING SEASON

The histological data on the histological methods employed in the present investigation of ovotestis during breeding season are recorded in Table No.9. The histological localization of different ovotesticular cells in the ovotestis of C semirugata is photomicrographically illustrated in Plate No.4, Figs. 1 to 8.

Histological staining reactions with HE1, HE2, MT and Feulgen staining methods were same as described in the histological observations on the ovotesticular cells of S. maculata, except few differences. The size of the follicles of this snail was smaller than those of the slug S.maculata. The follicles were placed away from the each other whereas in case of slugs those were placed very nearer to each other. The size of the cells associated with female sex cells i.e. ova and nurse cells were comparatively very large but the cells

Table 8 : The Numerical Changes in Neurosecrettory Cells in the Various Regions of cerebral Ganglion of Cryptozona semirugata During Breeding Season

Phase of the breeding season	Region of cerebral ganglion	CELL TYPES						
		TYPE I	TYPE II	TYPE III	TYPE IV	TYPE V	TYPE VI	TYPE VII
1 Early Breeding	DM	1	1	2	6	6	14	6
	DL	1	1	2	2	2	4	10
	VM	2	10	12	8	12	16	16
	VL	1	1	2	2	2	2	12
2 Mid Breeding	DM	1	1	4	6	8	16	6
	DL	1	1	2	2	2	4	10
	VM	2	10	12	12	16	18	20
	VL	1	1	2	2	2	2	18
3 Late Breeding	DM	1	1	4	6	6	14	16
	DL	1	1	2	2	2	4	10
	VM	2	10	12	10	12	16	18
	VL	1	1	2	2	2	2	16

related with the male sex cells i.e. spermatocytes, spermatids, sperms and Sertoli cells were small in size than those of the S.maculata.

In general, the staining reactivities of all the cellular elements in this snail were very much intense (Plate No.4, Figs. 2, 3,4,5,7 and 8). The number of these cells and their staining reactivity started increasing in early breeding period, reached their maximum level in mid-breeding period and reduced to the moderate level in the late breeding period.

D) HISTOCHEMICAL OBSERVATIONS ON OVOTESTIS DURING BREEDING SEASON. :

The histochemical data of some of the histochemical staining techniques employed in the present investigation of ovotestis during breeding season are recorded in Table No.9. The histochemical distribution of chemical constituents in different cells of the ovotestis of C. semirugata are photomicrographically illustrated in Plate No.4, Figs. 1 to 8.

As in case of S.maculata, glycogen and neutral mucosubstances were present in the germinal epithelial cells, nutritive cells, Sertoli cells and in sperm tails of C.semirugata. Similarly, acidic mucopolysaccharides, hyaluronic acid and sialic acid were present in the acrosomal head portion of the sperms and cortical zone of the ova, respectively.

The concentrations of these chemical constitutents in various cells of the ovotestis were at low levels in the early breeding period, which increased to their maximum levels in mid-breeding period. They reached again to the lowest levels

Table 9 : Histological and Histochemical Staining Reactions of Ovotesticular Cells of *Cryptozona semirugata* During Breeding Season

Name of the technique	Phase of the Breeding season	OVOTESTICULAR CELL TYPES					
		Germinal epithelial cell	Nutritive cell	Sertoli cell	Spermatid	Sperm	Ovum
1 HE	Early Breeding	+++	+	++	++	++	++
	Mid Breeding	++++	+++	++++	++++	++++	++++
	Late Breeding	+++	+	+++	+	++	+++
2 MT	Early Breeding	+++	++	++	++	++	++
	Mid Breeding	++++	+++	++++	++++	++++	++++
	Late Breeding	+++	+	+++	+	++	+++
3 PAS	Early Breeding	+++	++	++	++	++	++
	Mid Breeding	++++	++++	++++	++++	++++	++++
	Late Breeding	+++	+	+++	+	++	+++
4 AF	Early Breeding	-	-	-	-	-	-
	Mid Breeding	-	-	-	-	-	-
	Late Breeding	-	-	-	-	-	-
5 AB pH 2.5	Early Breeding	-	-	+	+	+	+
	Mid Breeding	-	-	+++	++	++++	++++
	Late Breeding	-	-	++	+	++	++
6 AB pH 1.0	Early Breeding	-	-	-	-	-	-
	Mid Breeding	-	-	-	-	-	-
	Late Breeding	-	-	-	-	-	-

N.B. : ++++ = Very intense reaction, +++ = Intense reaction, ++ = Moderate reaction, + = Poor reaction, - = No reaction.

Abbreviations : PAS = Periodic Acid Schiff, AB= Alcian Blue 8G X 300.

steadily upto the beginning of the aestivation period. The increase and decrease in the chemical constituents were not uniform in all the cells but varied in each cell type and in different phases of the breeding season.

Interestingly the increase and decrease in the chemical constituents of the ovotesticular cells were parallel with the increase and decrease in their number and also with the increase and decrease in the neuroscetory cells and in the concentrations of their secretory products in the cerebral ganglia.

E) HISTOLOGICAL AND HISTOCHEMICAL OBSERVATIONS ON NEUROSECRETORY CELLS IN OPTIC TENTACLES. :

In this species the histological structure of optic tentacles showed the epithelial lining on the inner surface of both the borders (inner and outer) in which scattered neurosecretory cells were interspreded. the distribution of these cells in the optic tentacles of C.semirugata during breeding season is photomicrographically illustrated in Plate No.6, Figs. 5 and 6. They were deeply stained with histological staining procedures.

All the histochemical staining techniques employed in the present investigation to show neurosecretory cells showed positive reactions . There cells could be clearly visible by their staining reactivities with these methods (Plate No.6, Figs. 5 and 6).

F) HISTOLOGICAL OBSERVATIONS ON CEREBRAL GANGLIA DURING AESTIVATION PERIOD :

The histological data on some of the histological staining methods employed in the present investigation of cerebral ganglia during aestivation are recorded in Table No.10. The histological distribution of neurosecretory cells in the cerebral ganglia of C.semirugata is photomicrograpically illustrated in Plate No.5, Figs. 1 and 6.

During aestivation period the size and number of the neurosecretory cells were decreased considerably, in every four regions of the cerebral ganglion. The staining reactivity of nucleus was reduced (Plate No.5, Fig.6).

The variations in the number of the seven types of cells during aestivation have been recorded in Table No.11. From the observations it seemed that the number of neurosecretory cells were at their moderate levels in the early aestivation period and reached at the minimum level during active aestivation period and these cells were without neurosecretory granules. The number of these cells started again increasing in the late aestivation phase.

G) HISTOCHEMICAL OBSERVATIONS ON CEREBRAL GANGLION DURING AESTIVATION PERIOD :

The histochemical data on some of the important histochemical staining techniques employed in the present investigation of cerebral ganglia during aestivation are recorded in Table No.10. The distribution of neurosecretory cells and their histochemical staining reactivities in the cerebral ganglia of

Table 10 : Histological and Histochemical staining reactions of neurosecretory cells in the cerebral ganglion of Cryptozona semirugata during aestivation

Name of the Technique	Organelles	CELL TYPES						
		TYPE I	TYPE II	TYPE III	TYPE IV	TYPE V	TYPE VI	TYPE VII
Histological								
HE 1	N	B ++	B +	B +	B +	B +	B +	-
	C	P ++	P +	P +	P +	P ±	-	-
HE 2	N	B ++	B +	B +	B +	B +	B +	-
	C	P ++	P +	P +	P +	P ±	-	-
MT	N	R ++	R +	R +	R +	R +	R +	-
	C	B ++	B +	B +	B +	-	-	-
Histochemical								
PAS	NG	M ++	M ++	M +	M +	-	-	-
Feulgen	N	M ++	M ++	M +	M +	M +	-	-
AF	NG	P ++	P ++	P ++	P +	P +	P ±	-
AB pH 2.5	NG	-	-	-	-	-	-	-
AB pH 1.0	NG	-	-	-	-	-	-	-
CHP	N	P +++	P +++	P ++	P ++	P +	P +	-
	NG	BL ++	BL ++	BL +	BL +	BL +	-	-

N.B. : ++++ = Very intense reaction, +++ = Intense reaction, ++ = Moderae reaction, + = Poor reaction , ± = Doubt full reaction, - = No reaction.

colours : B = Blue, P = Pink, R = Red, BL = Bluish black,

Abbreviations : N = Nucleus, C = Cytoplasm, NG = Neurosecretory granules, PAS= Periodic acid schiff, AB = alcian blue 8 G X 300.

Table 11 : The numarical changes in neurosertory cells in the various regions of cerebral ganglion of Crytozona semirugata during aestivation.

Phase in the aestivation		Region of cerebral ganglion	C E L L T Y P E S						
			TYPE I	TYPE II	TYPE III	TYPE IV	TYPE V	TYPE VI	TYPE VII
1	Early aestivation	DM	1	1	2	4	6	3	2
		DL	1	1	2	2	2	2	2
		VM	1	8	10	8	6	2	1
		VL	1	1	2	2	4	2	-
2	Mid aestivation	DM	1	1	2	2	4	2	-
		DL	1	1	2	2	2	1	-
		VM	1	8	7	5	3	1	-
		VL	1	1	1	2	2	-	-
3	Late aestivation	DM	1	1	2	4	4	1	-
		DL	1	1	2	2	2	2	-
		VM	1	6	12	10	14	4	1
		VL	2	1	2	2	2	4	2

C.semirugata during aestivation are photomicrographically illustrated in Plate No.5, Fig.2,3,4,5,7 and 8.

The histochemical data indicated over all decrease in staining reactivities of the neurosecretory cells and reduction in their number also. The decrease in the number of cells was in all the regions (DM,DL,VM and VL) of the cerebral ganglia. The decrease in the neurosecretory cells was different in early, mid and late aestivation periods.

H) HISTOLOGICAL AND HISTOCHEMICAL OBSERVATIONS ON THE OVOTESTIS DURING AESTIVATION PERIOD :

The histological and histochemical data of ovotesticular cells in C. semirugata during aestivation are recorded in Table No.12.

At a comparative level the staining reactivities of germinal epithelial cells, nutritive cells, Sertoli cells, ova, spermatids and sperms were decreased in the aestivation. The glycogen, neutral mucosubstances, hyaluronic acid and sialic acid were considerably decreased or some of the components were even absent during active aestivation in some of these cells. The histological and histochemical data of these ovotesticular cells are photomicrographically illustrated in Plate No.6, Figs. 1 to 4.

The decrease in the staining and in number of these cells were not uniform in three different phases of the aestivation period. It was moderate in early aestivation, lowest in the active aestivation and slightly increased in late

Table 12 : Histological and Histochemical Staining Reactions of Ovotesticular cells of Cryptozzona semirugata During aestivation

Name of the technique	Phase in the aestivation	OVOTESTICULAR CELL TYPES					
		Germinal epithelial cell	Nutritive cell	Sertoli cell	Spermatid	sperm	Ovum
HE	Early aestivation	++ P	+ P	+ P	+ P	+ P	+ P
	Mid aestivation	++ P	+ P	+ P	+ P	+ P	+ P
	Late aestivation	+ P	+ P	+ P	+ P	+ P	+ P
MT	Early aestivation	++ P	+ P	+ P	+ P	+ P	+ P
	Mid aestivation	++ P	+ P	+ P	+ P	+ P	+ P
	Late aestivation	+ P	+ P	+ P	+ P	+ P	+ P
PAS	Early aestivation	++ M	+ M	+ M	-	+ M	-
	Mid aestivation	++ M	+ M	+ M	-	+ M	-
	Late aestivation	+ M	+ M	+ M	-	+ M	-
AF	Early aestivation	-	-	-	-	-	-
	Mid aestivation	-	-	-	-	-	-
	Late aestivation	-	-	-	-	-	-
AB pH 2.5	Early aestivation	-	+ B	+ B	-	+ B	-
	Mid aestivation	-	+ B	+ B	-	+ B	-
	Late aestivation	-	+ B	+ B	-	+ B	-
AB pH 1.0	Early aestivation	-	-	-	-	-	-
	Mid aestivation	-	-	-	-	-	-
	Late aestivation	-	-	-	-	-	-

N.B. : ++++ = Very intense reaction, +++ = Intense reaction, ++ = Moderate reaction, + = Poor reaction, - = No reaction.

Abbreviations : PAS= Periodic Acid Schiff, AB = alcian blue 8 G X 300, P = Pink, M=Magenda, b = Blue.

aestivation period. These changes were parallel with the changes in the neurosecretory cells in the cerebral ganglion.

i) HISTOLOGICAL AND HISTOCHEMICAL OBSERVATIONS ON NEOROSECRETORY CELLS IN THE OPTIC TENTACLES DURING AESTIVATION :

The histological and histochemical data on some of the staining techniques employed in the present investigation of optic tentacles during aestivation are photomicrographically illustrated in Plate No.6, Figs. 7 and 8.

These staining reactivities and number of the neurosecretory cells in the optic tentacles decreased considerably when snail entered into the aestivation period. These variations occured steadily from early aestivation to active aestivation period. The number and staining reactivities again started increasing in the late aestivation period. These changes were also parallel with the similar changes in the neurosecretory cells of the cerebral ganglia of this snail.