III OBSERVATIONS

I. PROSTATE GLAND

i) Histological Observations :

A) Histological Observations on Normal Prostate Gland

The histological structure of the prostate gland of S. maculata visualised in the H.E. technique showed a number of polygonal lobules embeded in the thin connective tissue (Plate No.2, Fig.No.1). Each lobule consisted of a group of glandular cells, arranged radially around a small central lumen. The size of the lumen differed from lobule to lobule and even from season to season. Some lumens contained secretory material packed in them, while the others were empty.

The central lumen was bounded by thin cuboidal epithelial cells. These cells possessed cilia towards the luminal side. The glandular cells were columnar in shape having conspicuous nucleus with strongly hematoxylinophilic nucleus situated basally. On the basis of their secretory activity, the glandular cells could be differentiated into two types - one secretory cells with granular cytoplasm with closely packed innumerable yellowish-brown

Captions to Figures

PLATE NO. 2

(Histochemical alterations in the mucosubstances of prostate gland in S. maculata under the effects of hormones)

Fig. 1 : T.S. of normal prostate gland of control slug, stained with PAS, showing few follicles. Note secretory cells (SC), non-secretory cells (NSC) and lumens (L) filled with secretion. X 100.

Fig. 2 : T.S. of prostate gland taken on the 25th day after ablation of optic tentacles. Note reduced size, staining intensity of PAS in secretory (SC) and non-secretory (NSC) cells and shrunken lumens (L). X 100.

Fig. 3 : T.S. of normal prostate gland of control slug, stained with PAS showing secretory cells (SC), non-secretory cells (NSC) and lumen (L) in few follicles. X 100.

Fig. 4 : T.S. of prostate gland of optic tentacle extract injected slug with intact optic tentacles, taken on 20th day after treatment. Note increased number and staining intensity in secretory cells (SC) and non-secretory cells (NSC), most of the lumens (L) are empty. X 100.

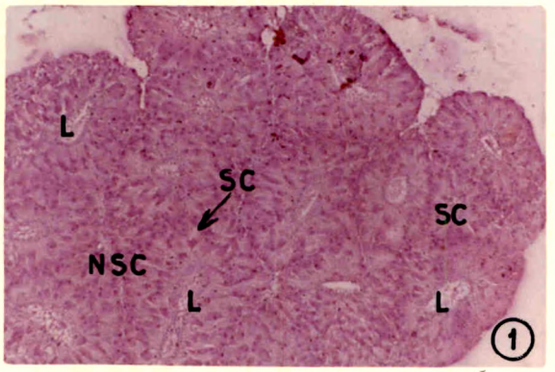
Fig. 5 : T.S. of normal prostate gland with usual structure of their secretory cells (SC), non-secretory cells (NSC) and Lumens (L). X 100.

Fig. 6 : T.S. of prostate gland of cerebral ganglia extract injected slug having intact optic tentacles, taken on 25th day after treatment and stained with PAS. Note increased staining of PAS in secretory cells (SC), non-secretory cells (NSC) and lumens (L). X 100.

Fig. 7 : T.S. of normal prostate gland showing few follicles with secretory (SC) and nonsecretory cells (NSC) with lumens (L). X 100.

Fig. 8 : T.S. of prostate gland of ovotestis extract injected slug, stained with PAS showing enhanced staining of PAS in secretory cells (SC), non-secretory cells (NSC) and also in the secretory products in the lumens (L). X 100.

PLATE No. 2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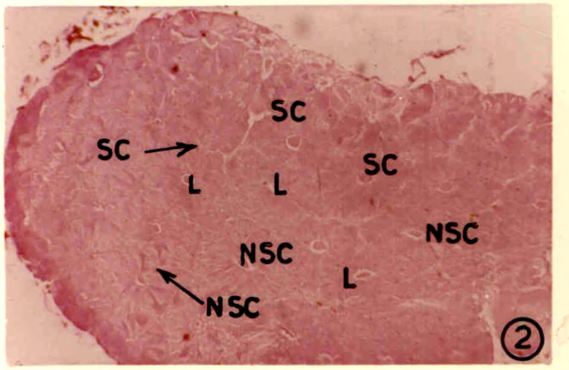
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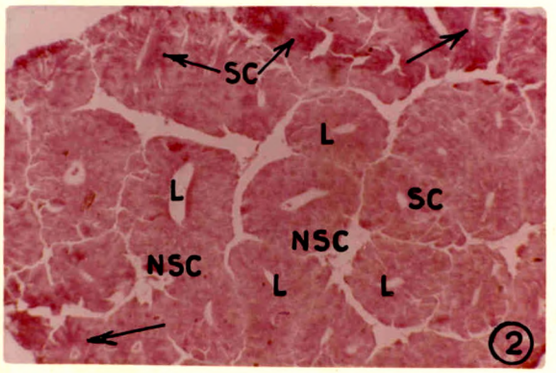
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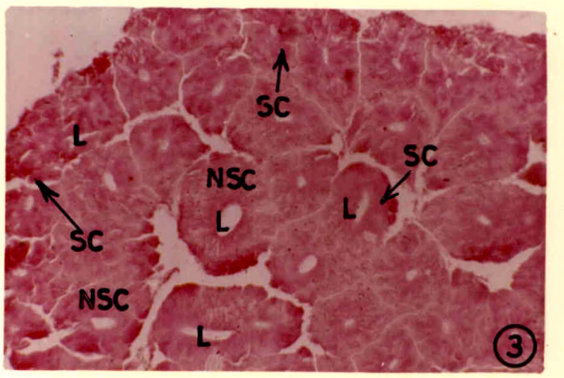
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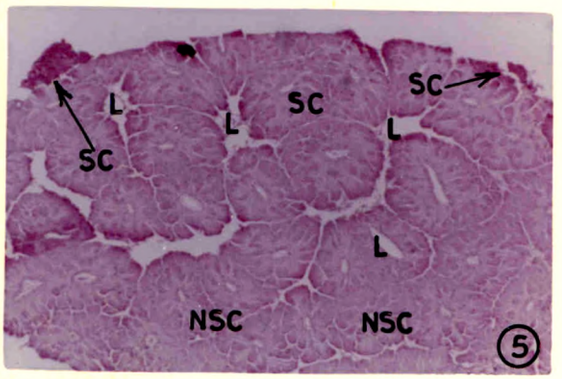
3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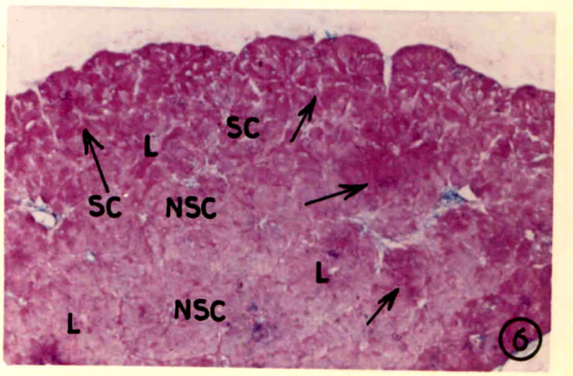
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NSC
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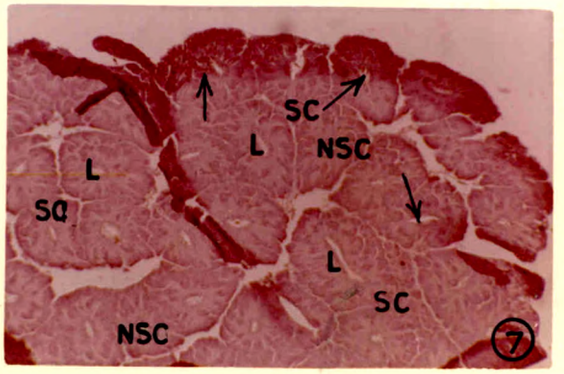
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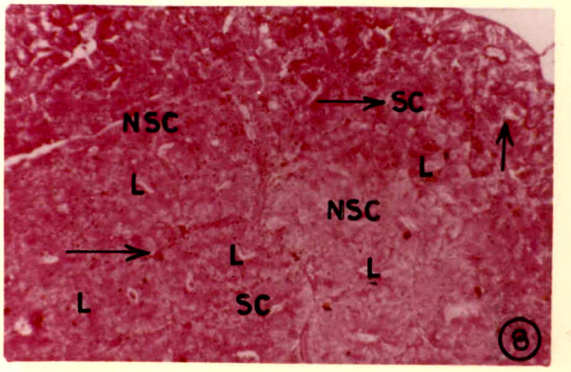
7


NSC
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granules, the other with no secretory activity. These two types of cells showed no difference in their structure, but their staining reactivity differed towards different histochemical and histological techniques.

Both types of cells showed cytoplasmic network in the entire body and they were unevenly interspaced between one another. These two types of cells were easilly recognizable from their structural features also. The secretion of these two types of cells stained differentially in H-E. The secretion in the secretory cells stained deep blue and that of the non-secretory cells stained red with H-E staining technique.

B) Effects of optic tentacular neurohormones on the histology of prostate gland :

The effects of neurohormones elaborated by the optic tentacles were observed by performing three experiments - (1) By ablation of optic tentacles, (2) After injections of extracts of optic tentacles to the slugs with ablated optic tentacles, and (3) After injections of extracts of optic tentacles to the slugs with intact optic tentacles.

The alterations in the histological structure were observed in all the three experiments. These changes were concerned with the size, the staining intensity and the staining characteristics of the epithelial cells.

In the first experiment the size of the cells and their staining intensity were reduced. On the other hand in the second and third set of experiments these parameters were increased.

C) Effects of cerebral ganglionic neurohormones on the histology of prostate gland :

The neurohormones elaborated by the cerebral ganglia also affected the histological structure of the prostate gland. In order to assess these effects of the neurohormones, another two sets of experiments were performed. (1) In first experiment, the extract of cerebral ganglia was injected to the slugs with ablated optic tentacles. (2) In second experiment, the same extract was injected to the slugs with intact optic tentacles.

In both the experiments there was an increase in the size of the epithelial cells, increase in the intensity of staining and concentration of secretory material in the epithelial cells. But these effects were comparatively less in the former group of slugs than the latter.

D) Effects of ovotesticular hormones on the histology of prostate gland :

The hormones elaborated by the ovotestis proper showed tremendous effects on the histological structure of this gland. These effects were assessed by injecting the extract of ovotestis to the slugs with and without optic tentacles. In both the cases, there were

stimulatory effects in the prostate gland. The size of the cells was increased and the staining intensity was reached to its maximum level. But these effects were more in the slugs with intact tentacles as in case of experiments involving the cerebral ganglia extract.

ii) Histochemical observations :

A) Elaboration of mucopolysaccharides by the normal prostate gland :

The glandular epithelial cells of the prostate gland elaborate typical mucopolysaccharide substances. On the basis of their reactivities with histochemical staining techniques, the cells could be differentiated into two types : one secretory cells and other non-secretory cells.

The histochemical data on some important staining reactions employed in the present investigation of the prostate gland are recorded in Table No.1 according to the visually estimated intensity and shade with four plus (++++) representing the strongest activity. The distribution of mucopolysaccharides in this gland are illustrated photo-micrographically in Figs. 1, 3, 5, 7 of Plate No.2 and Plate No.3. The histochemical results requiring further description and consideration are presented hereafter along with the interpretations of the histochemical staining reactions.

Table No.1 : Histochemical staining reactions of the **prostate gland** of S. maculata

Sr.No.	Histochemical Techniques	Tissues of the prostate gland				
		Secretory cells (SC)	Non-secretory cells (NSC)	Connective tissue (CT)	Luminal contents (LC)	
1.	PAS	+++P	++P	++P	+++P	++P
2.	Saliva digestion -PAS	+++P	-	++P	+++P	-
3.	AB pH 1	-	-	-	-	-
4.	AB pH 2.5	± B	-	++B	+B	-
5.	AB pH 1-PAS	+++P	++P	++P	+++P	++P
6.	AB pH 2.5-PAS	+++P	++P	++B	+++P	++P

Secretory Cells :

The secretion of these cells were in the granular form. These cytoplasmic granular contents exhibited an intense PAS reactivity (Plate No.2, Fig.1,3,5,7) which was resistant to prolonged saliva digestion. These cells did not exhibit any alcianophilia both at pH 1 and pH 2.5. In the combined sequential staining with AB (pH 1)-PAS and AB(pH 2.5)-PAS, they showed no trace of blue colouration. These histochemical reactions indicate that these cells are endowed with a capacity to elaborate only neutral mucosubstances.

Non-Secretory Cells :

The cytoplasmic contents of these cells exhibited moderate PAS reactivity, which was completely abolished by saliva digestion. These cells reacted negatively with the rest of the histochemical alcianophilic techniques employed. In the combined sequential staining with AB (pH 1, 2.5)-PAS, these cells stained only with PAS giving a pink colouration, there being no trace of blue staining. The above histochemical reactions indicate that these cells are endowed with a capacity to elaborate only glycogen in them. At a comparative level these cells were more in number than the secretory cells.

Luminal contents :

The luminal contents showed both the types of reactions just described for the above two types of cells, indicating the presence of neutral mucopolysaccharides and glycogen in them.

B) Effects of optic tentacular neurohormones on the elaboration of mucopolysaccharides of prostate gland :

The histochemical data on some important staining reactions employed in the present investigation of the glandular cells of the prostate gland and on the distribution and alterations in their mucopolysaccharides under the influence of various hormones elaborated by the optic tentacles, cerebral ganglia and ovotestis are recorded in Table No.2 and also illustrated photomicrographically in Plate No.2, Figs.1 to 8 and Plate No.3, Figs. 1 to 8.

The ablation of bilateral optic tentacles showed interesting changes in the intensity of staining of the mucopolysaccharides in the two types of cells and in the luminal contents. Curiously these changes consisted not only in the intensity of staining but also in the number of the two types of cells and in the luminal contents.

The secretory cells showed minimum PAS staining intensity (Plate No.2, Fig.2). The alcianophilic staining reactivity of these cells was totally lost (Plate No.3, Fig.2). The non-secretory cells also showed minimum PAS staining intensity (Plate No.2, Fig.2). These results indicated the presence of minimum concentration of

Table No.2 : Histochemical staining reactions of **prostate gland** in S. maculata under the influence of various hormones of optic tentacles, cerebral ganglia and ovotestis.

Sr. No.	Histochemical Techniques	Tissues of Prostate gland	Control Group	Experimental conditions						
				OTAB	OTAB + OTEI	OTIN + OTEI	OTAB + CGEI	OTIN + CGEI	OTAB + OVEI	OTIN + OVEI
1.	PAS	SC	+++P	+P	++++P	++++P	+++P	++++P	++++P	++++P
		NSC	++P	-	+++P	+++P	+++P	++++P	+++P	++++P
		LC	+++P	+P	++++P	++++P	+++P	++++P	++++P	++++P
2.	Saliva diges-tion - PAS	SC	+++P	+P	++++P	++++P	+++P	++++P	++++P	++++P
		NSC	-	-	-	-	-	-	-	-
		LC	+++P	+P	++++P	++++P	+++P	++++P	++++P	++++P
3.	AB pH 1	SC	-	-	-	-	-	-	-	-
		NSC	-	-	-	-	-	-	-	-
		LC	-	-	-	-	-	-	-	-
4.	AB pH 2.5	SC	±B	-	++B	+++B	++B	+++B	+++B	++++B
		NSC	-	-	-	-	-	-	-	-
		LC	+B	-	++B	+++B	++B	+++B	+++B	++++B
5.	AB pH 1-PAS	SC	+++P	+P	++++P	++++P	+++P	++++P	+++P	++++P
		NSC	++P	-	+++P	+++P	+++P	++++P	+++P	++++P
		LC	+++P	+P	++++P	++++P	+++P	++++P	+++P	++++P
6.	AB pH 2.5-PAS	SC	+++P	+P	+B	+++B	++B	+++B	+++B	++++B
		NSC	++P	-	-	-	-	-	-	-
		LC	+++P	+P	++B	+++B	+B	+++B	+++B	++++B

neutral mucopolysaccharides in the secretory cells and minimum level of glycogen in the non-secretory cells.

On the contrary, after injections of extracts of optic tentacles revealed the reverse effects. The PAS staining reactivity of both the cell types was enhanced (Plate No.2, Fig.4). The moderate alcianophilic staining reactivity of secretory cells was increased and showed intense alcianophilia at pH 2.5 (Plate No.3, Fig.4) indicating conversion of neutral mucopolysaccharides into acidic mucopolysaccharides. The glycogen content in the non-secretory cells was also increased. These effects were more pronounced in the slugs with intact optic tentacles than those of ablated optic tentacles.

C) **Effects of cerebral ganglionic neurohormones on the elaboration of mucopolysaccharides of prostate gland** :

The alterations in the elaboration of mucopolysaccharides in the various cellular elements of the prostate glands due to cerebral ganglionic neurohormones have been recorded in Table No.2 and also illustrated photomicrographically in Fig.6 of Plate No.2 and Plate No.3.

The effects are similar to those seen after the injections of extracts of optic tentacles. They showed increased intensity of staining reactivity, increase in number of both the cell types, increase in the concentration of mucopolysaccharides contents in

Captions to Figures

PLATE NO. 3

(Histochemical alterations in the mucosubstances of prostate gland in S. maculata under the effects of hormones)

Fig. 1 : T.S. of normal prostate gland of control slug, stained with AB (pH 2.5)-PAS, showing alcianophilic secretory cells (SC) and PAS positive non-secretory cells (NSC) and lumens (L) in few follicles. X 100.

Fig. 2 : T.S. of prostate gland taken on 25th day from the slugs with ablated optic tentacles. Note loss of alcianophilia and PAS positivity in the secretory (SC) and non-secretory cells (NSC). Lumens are without secretion. X 100.

Fig. 3 : T.S. of normal prostate gland of control slug, stained with AB pH 2.5-PAS. Note secretory (SC), non-secretory (NSC) cells and lumens (L). X 100.

Fig. 4 : T.S. of prostate gland, stained as in Fig.3, from the optic tentacles extract injected slugs with intact optic tentacles taken on 25th day after injections. Note increased staining in secretory (SC), non-secretory cells (NSC) and in lumens (L). X 100.

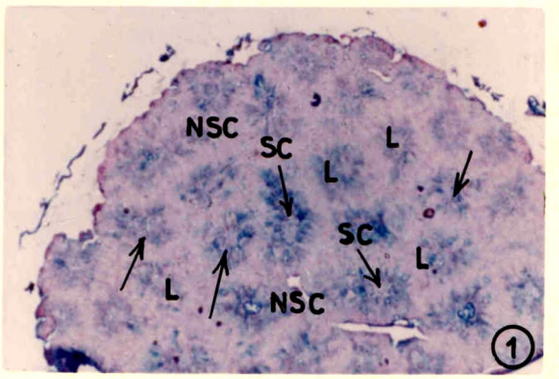
Fig. 5 : T.S. of prostate gland of control slug stained with AB pH 2.5-PAS, showing secretory (SC), non-secretory cells (NSC) and lumens (L). X 100.

Fig. 6 : T.S. of prostate gland stained as in Fig.5 showing increased staining in secretory (SC), non-secretory cells (NSC) and in lumens (L) in slugs with optic, tentacles and injected with cerebral ganglia extract X 100.

Fig. 7 : T.S. of prostate gland of control slug, stained in AB pH 2.5-PAS, showing secretory cells (SC), non-secretory cells (NSC) and lumens (L). X 100.

Fig. 8 : T.S. of prostate gland stained as in Fig.7 taken from the slugs with intact optic tentacles and injected with the ovotestis extract. Note enhanced staining in secretory (SC) and non-secretory cells (NSC) and in lumens (L). X 100.

PLATE No. 3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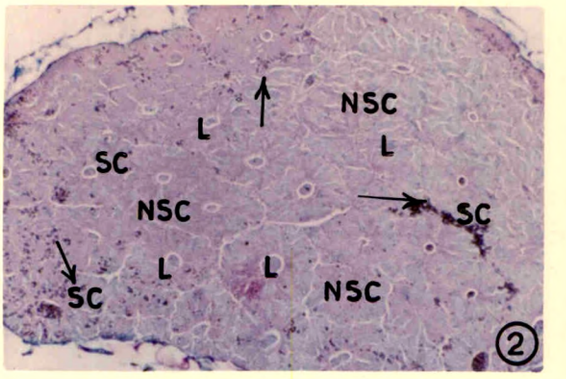
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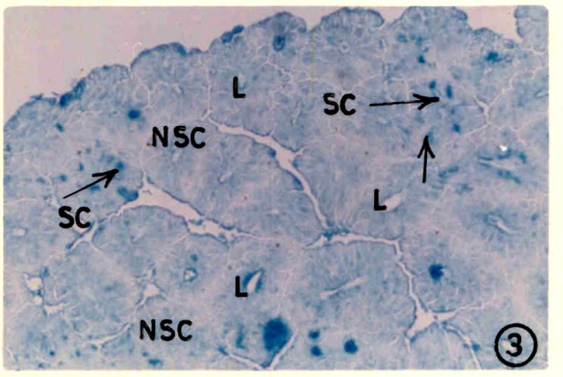
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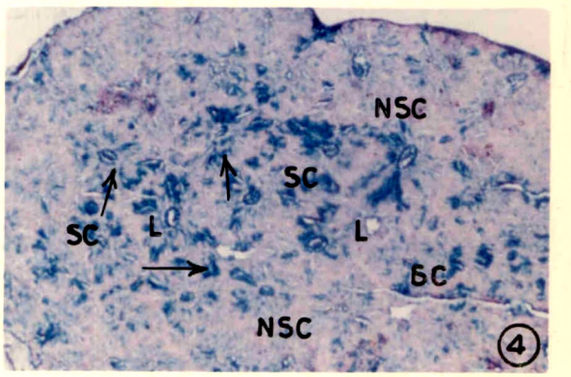
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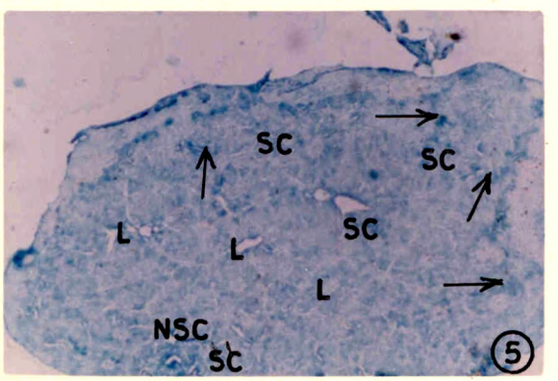
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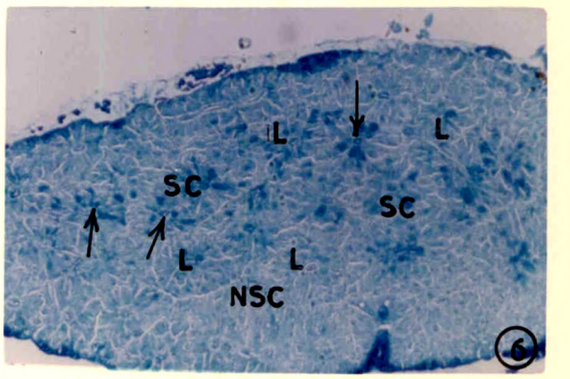
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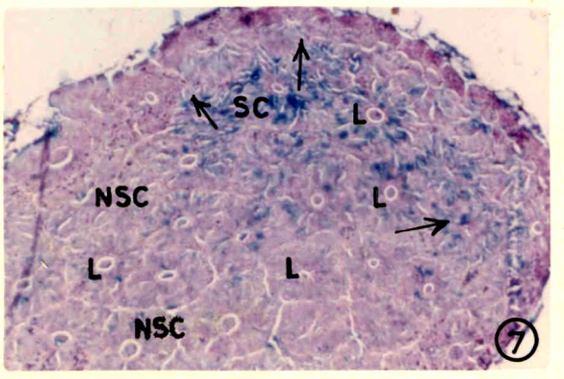
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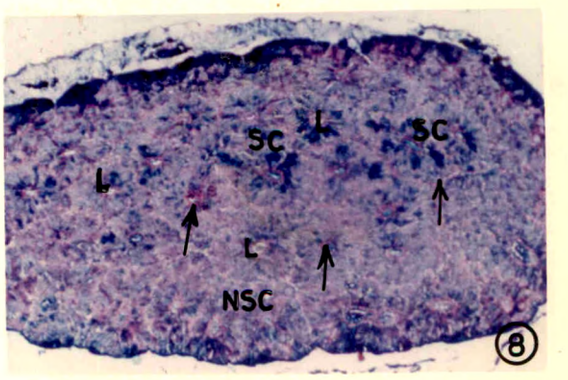
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the cells and change from neutral to acidic mucopolysaccharides in the secretory cells. These effects were more in the slugs with intact optic tentacles than those with the ablated optic tentacles.

D) Effects of ovotesticular hormones on the elaboration of mucopolysaccharides of prostate gland :

The injections of the extracts of ovotestis produced alterations in the elaboration of mucopolysaccharides in the prostate gland. These changes were very intense but similar to those observed after the injections of extracts of optic tentacles and cerebral ganglia. The concentration, the staining intensity and number of mucopolysaccharide secreting cells were maximum after this treatment (Plate No.2, Fig.6 and Plate No.3, Fig.6). It indicated that the ovotesticular hormones stimulated the elaboration of mucopolysaccharides and interconversion of neutral to acidic mucopolysaccharides and glycogen synthesis in the both the cells of the prostate gland.

iii) Biochemical observations :

A) Biochemical observations on the glycogen, proteins and cholesterol in the normal prostate gland :

The glycogen, proteins and cholesterol per cent values in the prostate gland of control slugs are recorded in Table No.3. The average concentration of glycogen, proteins and cholesterol was

Table No.3 : Comparative data on variations in the glycogen, proteins and cholesterol in **prostate gland** of S. maculata under the influence of hormones of optic tentacles, cerebral ganglia and ovotestis.

Sr. No.	Experimental conditions	Nutrients	VALUES DURING EXPERIMENTAL DAYS 5	10	15	20	25
1	Control	Glycogen	7.57 ± 1.02	8.05 ± 1.00	8.56 ± 0.62	8.20 ± 0.22	8.12 ± 0.50
		Proteins	7.27 ± 1.03	7.87 ± 0.74	8.00 ± 0.50	8.01 ± 0.87	8.03 ± 0.25
		Cholesterol	5.50 ± 1.23	6.56 ± 0.88	6.98 ± 0.33	7.60 ± 0.65	7.34 ± 0.63
2	OTAB	Glycogen	6.57 ± 1.00	5.63 ± 0.27	4.78 ± 0.88	4.01 ± 0.34	2.87 ± 0.74
		Proteins	6.41 ± 1.01	5.00 ± 0.25	4.01 ± 0.37	3.00 ± 0.42	2.58 ± 0.81
		Cholesterol	4.50 ± 0.50	4.01 ± 0.17	3.67 ± 0.61	2.50 ± 0.25	2.69 ± 0.64
3	OTAB+OTEI	Glycogen	9.02 ± 1.00	11.53 ± 1.50	12.49 ± 0.36	13.54 ± 0.97	14.20 ± 0.22
		Proteins	8.55 ± 1.20	11.14 ± 1.25	12.50 ± 0.59	12.25 ± 0.24	12.52 ± 0.94
		Cholesterol	10.00 ± 1.00	11.05 ± 1.30	13.80 ± 1.02	14.50 ± 0.99	14.00 ± 0.66
4	OTIN+OTEI	Glycogen	11.20 ± 1.00	13.82 ± 1.00	14.00 ± 1.05	13.35 ± 0.31	14.30 ± 0.43
		Proteins	9.25 ± 1.03	14.33 ± 1.04	15.43 ± 1.00	15.52 ± 0.57	16.00 ± 0.69
		Cholesterol	10.52 ± 1.00	11.81 ± 0.87	12.56 ± 0.98	13.11 ± 0.43	16.78 ± 0.56
5	OTAB+CGEI	Glycogen	10.76 ± 1.00	11.34 ± 0.87	12.85 ± 0.62	13.00 ± 0.73	15.52 ± 0.98
		Proteins	9.47 ± 0.50	10.63 ± 0.26	11.21 ± 0.42	12.61 ± 0.23	13.21 ± 0.08
		Cholesterol	7.80 ± 0.02	8.52 ± 0.33	10.00 ± 0.75	11.55 ± 0.69	12.56 ± 0.44
6	OTIN+CGEI	Glycogen	11.35 ± 2.00	12.87 ± 0.87	13.50 ± 0.47	14.65 ± 0.74	16.42 ± 0.87
		Proteins	10.23 ± 1.00	12.11 ± 0.90	14.63 ± 0.88	15.21 ± 0.33	16.62 ± 0.56
		Cholesterol	9.80 ± 0.50	11.80 ± 0.59	13.50 ± 0.35	14.63 ± 0.41	15.00 ± 0.97
7	OTAB+OVEI	Glycogen	9.46 ± 0.50	10.32 ± 0.61	11.73 ± 0.19	12.86 ± 0.24	13.80 ± 0.24
		Proteins	7.54 ± 0.25	8.87 ± 0.72	10.04 ± 0.62	11.34 ± 0.17	12.63 ± 0.55
		Cholesterol	6.20 ± 0.25	8.52 ± 0.72	10.63 ± 0.62	12.00 ± 0.20	13.63 ± 0.26
8	OTIN+OVEI	Glycogen	10.30 ± 0.50	11.62 ± 1.00	12.86 ± 0.63	13.06 ± 0.90	14.73 ± 0.81
		Proteins	9.69 ± 0.63	10.64 ± 0.50	11.26 ± 0.32	12.63 ± 0.95	13.50 ± 0.74
		Cholesterol	8.80 ± 0.74	10.45 ± 0.25	12.04 ± 0.56	13.54 ± 0.74	14.30 ± 0.68

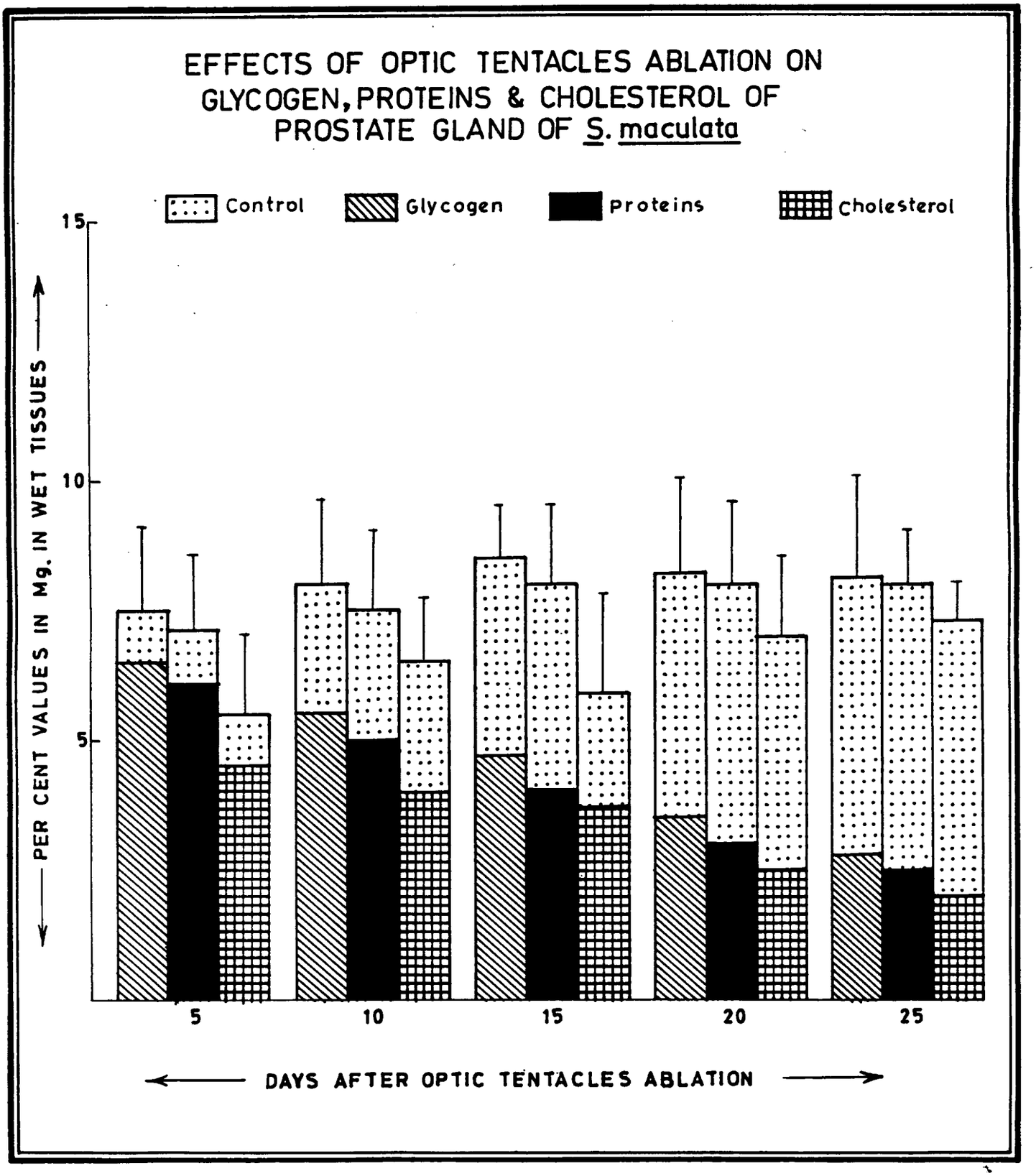
7.57 mg, 7.27 mg and 5.50 mg per cent wet tissue of prostate gland, respectively.

B) Effects of optic tentacular neurohormones on the glycogen, proteins and cholesterol of prostate gland :

The neurohormones elaborated in the optic tentacles altered the concentrations of glycogen, proteins and cholesterol of the prostate gland. These alterations are recorded in Table No.3 and they are shown graphically in Graph Nos.1 and 2.

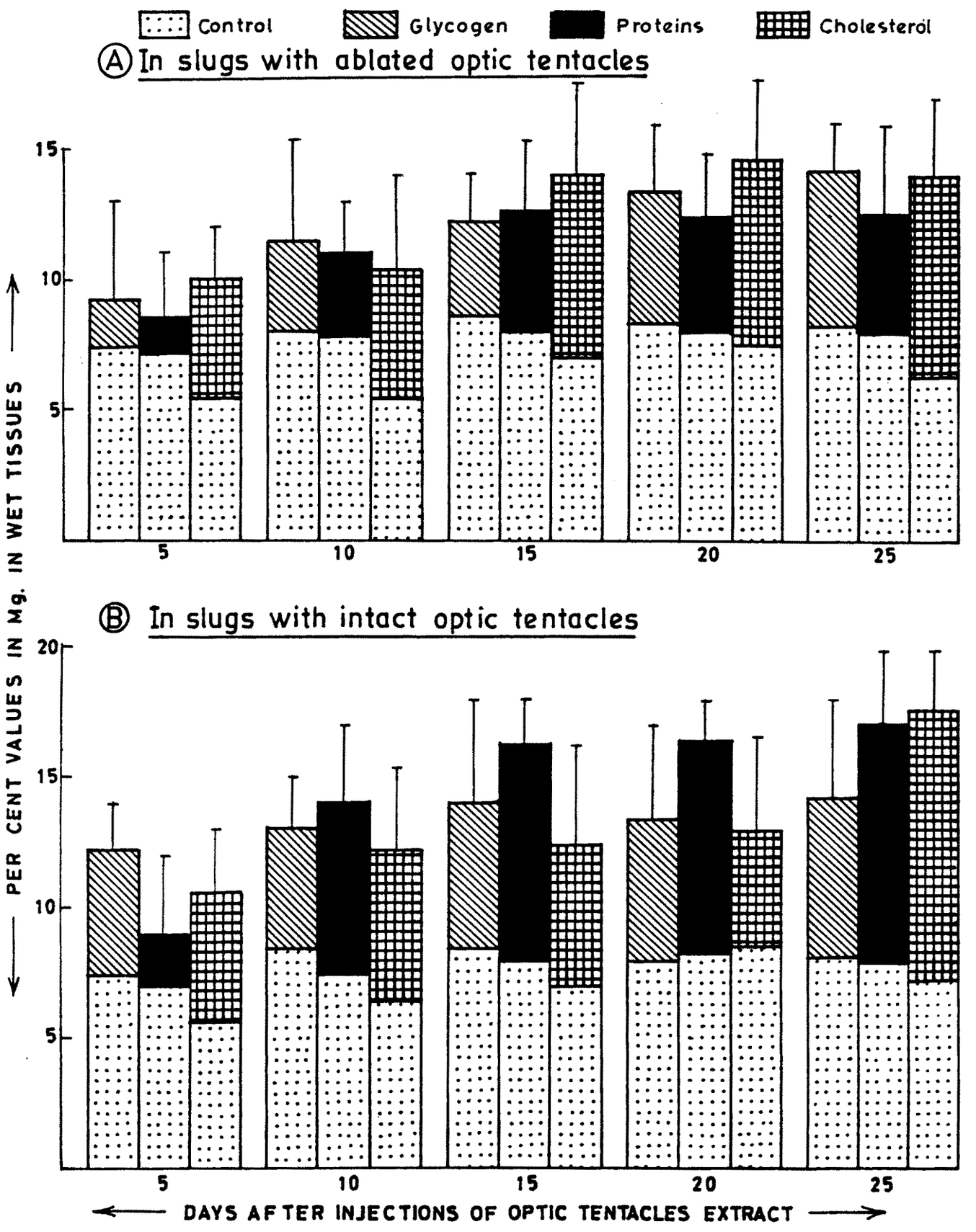
When the optic tentacles of slugs were ablated, the per cent values of glycogen, proteins and cholesterol started decreasing. On the 5th day these values were 6.57 mg, 6.12 mg and 4.50 mg, respectively. Through intermediate figures on 10th, 15th and 20th day these values droped upto 2.87 mg, 2.58 mg and 2.65 mg, respectively on the 25th day of ablation. Thus by stoping of supply of neurohormones by ablation of optic tentacles decrease in the concentrations of all these nutrients was observed.

On the other hand, when the injections of extracts of optic tentacles were given to the slugs the concentration of glycogen, proteins and cholesterol was increased from control values to 9.02 mg and 11.20 mg, 8.55 mg and 9.25 mg and 10.00 mg and 10.52 mg on 5th day in the slugs with ablated tentacles and in the slugs with intact tentacles, respectively. These values increased through intermediate values upto 14.20 mg and 14.30 mg, 12.52 mg and 16


EFFECTS OF OPTIC TENTACLES ABLATION ON
GLYCOGEN, PROTEINS & CHOLESTEROL OF
PROSTATE GLAND OF S. maculata
Control
Glycogen
Proteins
Cholesterol
15
10
5
PER CENT VALUES IN Mg. IN WET TISSUES
5
10
15
20
25
DAYS AFTER OPTIC TENTACLES ABLATION

GRAPH No.1

EFFECTS OF NEUROHORMONES IN OPTIC TENTACLES ON PROSTATE GLAND GLYCOGEN, PROTEINS & CHOLESTEROL OF S. maculata



GRAPH No. 2

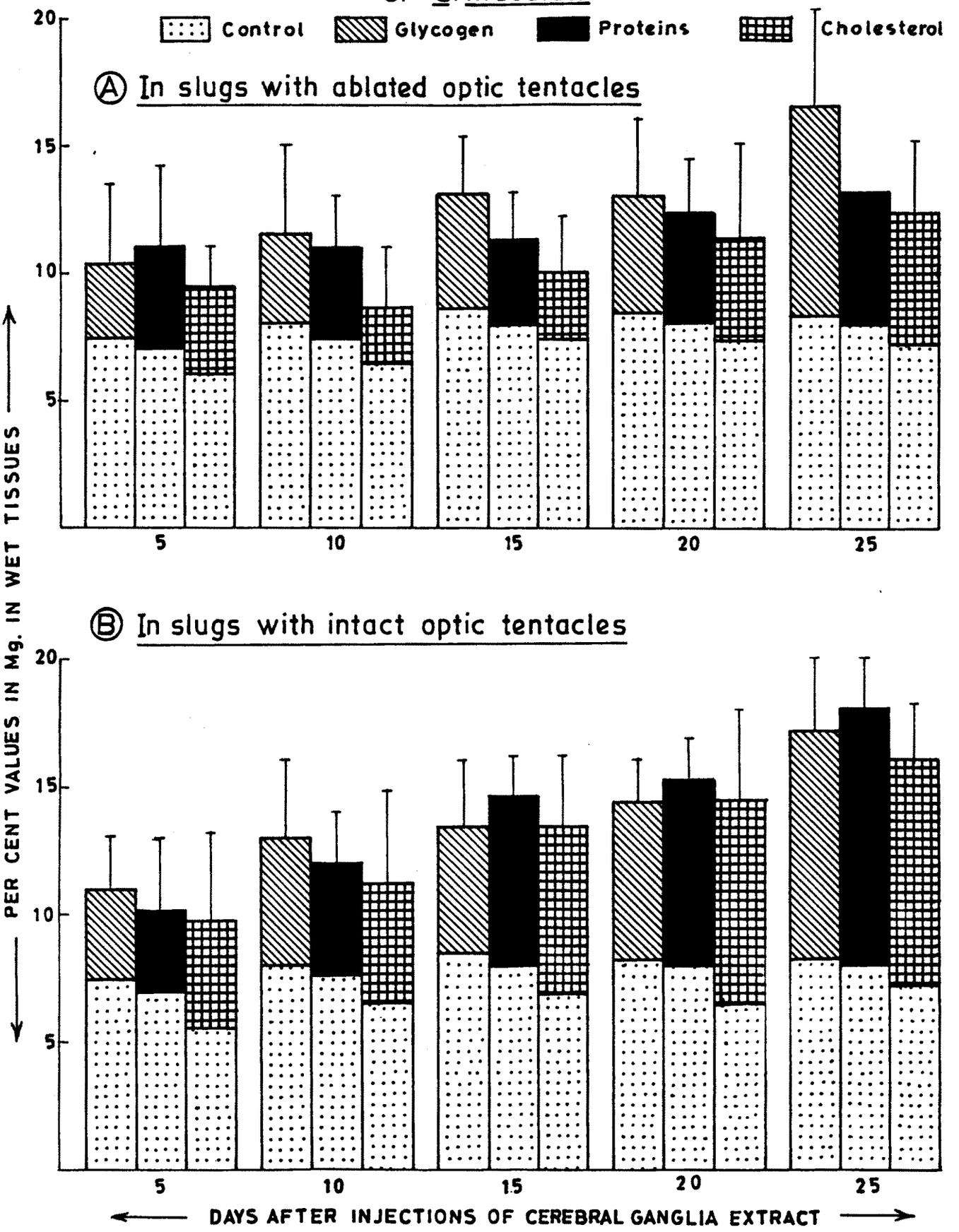
mg and 14 mg and 16.78 mg on 25th day in the slugs of both the groups, respectively. This indicated that the neurohormones stimulated the synthesis and storage of nutrients in the cells of the prostate gland.

C) **Effects of cerebral ganglionic neurohormones on the glycogen, proteins and cholesterol of prostate gland** :

The injections of the extracts of neurohormones elaborated in the cerebral ganglia to the slugs with ablated optic tentacles (OTAB +CGEI Group) and to the slugs with intact optic tentacles (OTIN +CGEI Group) increased the concentration and storage of glycogen, proteins and cholesterol in the various cells of the prostate gland. These changes are recorded in Table No.3 and they are also shown graphically in Graph No.3.

The glycogen per cent values in OTAB + CGEI and OTIN + CGEI groups were 0.76 mg,and 11.35 mg, 11.39 mg and 12.87 mg, 12.85 mg and 13.50 mg, 13 mg and 14.65 mg and 15.52 mg and 16.42 mg on 5th, 10th, 15th, 20th and 25th day in the above groups of slugs, respectively. The proteins per cent values in the both these groups were 9.47 mg and 10.23 mg, 10.63 mg and 12.11 mg, 11.21 mg and 14.65 mg, 12.61 mg and 15.21 mg and 13.21 mg and 16.62 mg 5th, 10th, 15th, 20th and 25th day, respectively. The cholesterol per cent values on these days in the both the groups were 7.80 mg and 9.80 mg, 8.52 mg and 11.80 mg, 10 mg and

EFFECTS OF NEUROHORMONES IN CEREBRAL GANGLIA ON PROSTATE GLAND GLYCOGEN, PROTEINS & CHOLESTEROL OF S. maculata



GRAPH No. 3

13.30 mg, 11.55 mg and 14.63 mg and 12.56 mg and 15 mg, respectively.

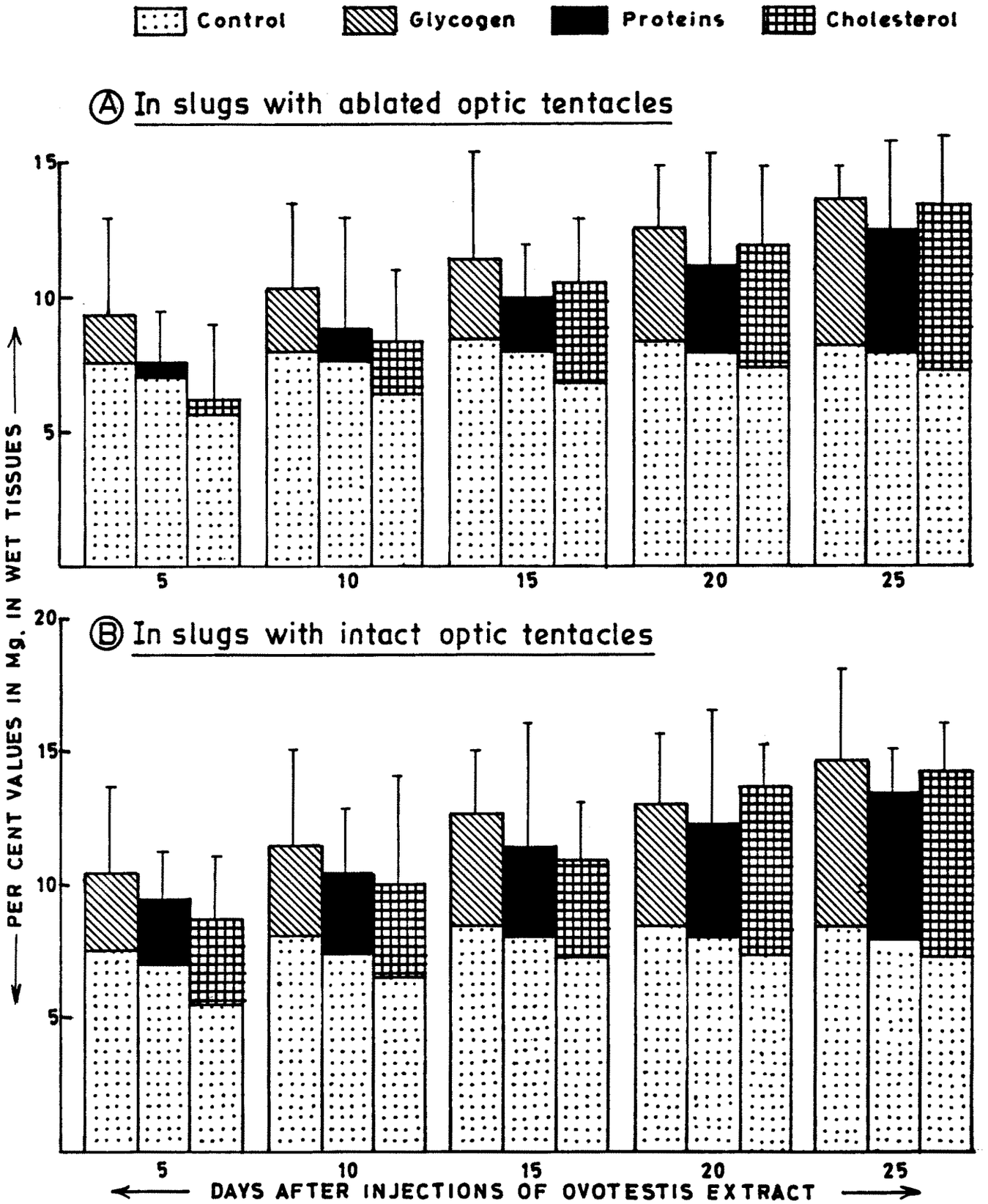
D) Effects of ovotesticular hormones on the glycogen, proteins and cholesterol of prostate gland :

The injections of the extract of ovotestis to the slugs with ablated optic tentacles and to the slugs with intact optic tentacles increased the concentration of glycogen, proteins and cholesterol in the prostate gland. These alterations are recorded in Table No.3 and they are shown graphically in Graph No.4.

The percentage of glycogen, proteins and cholesterol in the group of slugs with ablated tentacles was 9.46, 7.54, 6.20; 10.32, 8.87, 8.52; 11.73, 10.04, 10.63; 12.86, 11.34, 12 and 13.80, 12.63, 13.63 on 5th, 10th, 15th, 20th and 25th day, respectively. These values in the group of slugs with intact tentacles were 10.30, 9.69, 8.80; 11.52, 10.59, 10.45; 12.86, 11.26, 12.04; 13.05, 12.63, 13.54 and 14.73, 13.50, 14.30 on 5th, 10th, 15th, 20th and 25th day, respectively.

Thus, the results indicated that the neurohormones elaborated by the optic tentacles and cerebral ganglia and the hormones elaborated by the ovotestis stimulated the synthesis and storage of three nutrients i.e. glycogen, proteins and cholesterol in the various cells of the prostate gland of S. maculata.

EFFECTS OF HORMONES IN OVOTESTIS ON PROSTATE GLAND GLYCOEGEN, PROTEINS & CHOLESTEROL OF *S. maculata*



GRAPH No. 4

2. DART GLAND

i) Histological observations :

A) Histological observations on Normal Dart Gland :

There are 10 to 15 whitish thread like tubular projections clustered at the base of penis near its opening called as dart gland or multifed gland. The transverse sections looked more or less circular in shape with a large lumen at the center, containing amorphous watery fluid in which granular secretions in the form of droplets were embeded. The amorphous secretion in the lumen stained pinkish in H-E technique, whereas the droplets exhibited blue or bluish purple staining in this method. The histological structure visualised in the H-E staining technique.The glandular secretory cells were located peripherally, contained basal large oval nuclei. The cytoplasm of these cells stained mostly purplish in H-E technique. Some cells contained granular secretion which stained deep-blue in this method. In between the cells and towards the luminal border, a thick muscular band was present. This muscle band was perforated by columnar canallike cytoplasmic projections of the peripheral cells. There were small, oval but non-grandular epithelial cells bordering the lumen, contained cilia projecting in the lumen. They stained faintly pink in H-E technique.

B) Effects of optic tentacular neurohormones on the histology of dart gland :

The neurohormones altered the histological characteristics of the dart gland. The alterations were concerned with the size, staining intensity and staining characteristics of the peripheral glandular cells. When the neurohormones were stopped by ablation of optic tentacles these cells were shrunken, their size was considerably reduced and their staining intensity was decreased. The luminal diameters were reduced and some of lumens were emtpty.

On the other hand, when extract of optic tentacles was injected to the slugs, the size, staining intensities and staining characteristics of the glandular cells were increased. The lumens were filled with secretions. Their size was increased. The increase in these parameters was more in the group of slugs with intact optic tentacles than in the group of slugs with intact optic tentacles.

C) Effects of cerebral ganglionic neurohormones on the histology of dart gland :

The neurohormones of the cerebral ganglia altered the histology of the dart gland. The changes were similar to those observed under the influence of neurohormones of the optic tentacles and they were concerned with the change in size of the cells, staining intensities of the cells, increase in number of

glandular cells and increase in diameter and secretory material in the lumens of the tubules. The results indicated that the neurohormones of the cerebral ganglia stimulated the cellular differentiation, growth and synthesis of secretory products in the dart gland of S. maculata.

D) Effects of ovotesticular hormones on the histology of dart gland :

The hormones of the ovotestis also affected the histological architecture of the dart gland. Therefore, when the extract of ovotestis was injected to the slugs, the peripheral glandular cells of this gland were inlarged, they contained more granular secretion in their cytoplasm. The staining intensity of the cells were increased and the number of these cells was increased. The lumens were dialated and filled with secretory droplets. These effects were more pronounced in the slugs with intact optic tentacles than those observed in the slugs with ablated optic tentacles. This indicated that these hormones stimulated the growth, differentiation of cells and secretory activity of the dart gland.

ii) Histochemical observations :

A) Elaboration of mucopolysaccharides by the normal dart gland :

The histochemical data on some important staining reactions employed in the present investigation of the dart gland

are recorded in Table No.4, according to the visually estimated intensity and shade with four plus (++++) represeting the strongest activity. The distribution of mucopolysaccharides in this gland are illustrated photomicrographically in Figs.1,3,5,7 of Plate No.4 and Plate No.5. The histochemical results requiring further description and consideration are presented hereafter along with the interpretations of the histochemical staining reactions. The peripheral gladular cells showed elaboration of different types of mucosubstances. There are three significant facts about the elaboration of the mucosubstances by these cells-(1) on the basis of their histochemical reactivities, these cells could be classified into five types. (2) Broadly neutral polysaccharides, acidic polysaccharides and mixed (neutral + acidic) polysaccharides are synthesized by these different cell types, (3) Luminal secretory droplets embeded in the amorphous fluid exhibited neutral, acidic and mixed, mucopolysaccharide histochemical characterstics similar to those of glandular cells.

Peripheral glandular cells :

Neutral mucopolysaccharides secretory cells :

These cells exhibited an intense PAS reactivity, which was resistant to prolonged saliva digestion. They reacted negatively to AB (pH 1 and 2.5) staining techniques. In the combined sequential staining with AB (pH 1) - PAS and AB (pH 2.5)-PAS, these cells reacted only with PAS giving a pink staining, there being no trace

Table No.4 : Histochemical staining reactions of the **dart gland** of S. maculata

Sr. No.	Histochemical Techniques	TISSUES OF THE DART GLAND								
		Peripheral cells					Luminal contents			
		Glandular cells			Non-Glandular cells (NGC)	Muscular band (MB)	Amorphous (AS)	Secretory droplets		
		Neutral (NGC)	Acidic (AGC)	Mixed (MGC)				Neutral (NSD)	Acidic (ASD)	Mixed (MSD)
1.	PAS	+++P	+++P	++P	++P	+++P	+++P	+++P	+++P	++P
2.	Saliva digestion-PAS	+++P	+++P	++P	-	-	+++P	+++P	+++P	++P
3.	AB pH 1	-	+++B	++B	-	-	-	-	+++B	++B
4.	AB pH 2.5	-	+++B	++B	-	-	-	-	+++B	++B
5.	AB pH1 - PAS	+++P	+++B	++BP	++P	+++P	+++P	+++P	+++B	++BP
6.	AB pH 2.5-PAS	+++P	+++B	++BP	++P	+++P	+++P	+++P	+++B	++BP

Captions to Figures

PLATE NO. 4

(Histochemical alterations in the mucosubstances of dart gland in S. maculata under the effects of hormones)

Fig. 1 : T.S. of normal dart gland stained with PAS, showing extended tubules with peripheral glandular cells (PGC) and large lumens (L) with secretion. X 100.

Fig. 2 : T.S. of dart gland tubules stained with PAS of the slug with ablated its optic tentacles and taken on 25th day after ablation. Note reduction in the size of tubules and staining activity in peripheral glandular cells (PGC) and loss of staining in connective tissue and in lumens (L). X 100.

Fig. 3 : T.S. of normal dart gland tubules stained with PAS with usual peripheral glandular cells (PGC) and normal luminal (L) content. X 100.

Fig. 4 : T.S. of dart gland tubules stained with PAS of slug with intact optic tentacles and injected optic tentacles extract, taken on 25th day after injections. Note enhanced staining in luminal contents (LC) and tubular cells (PGC). X 100.

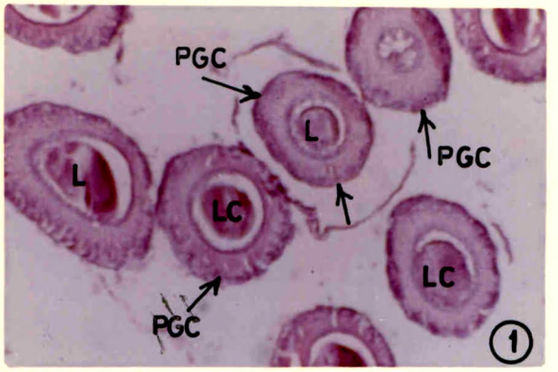
Fig. 5 : T.S. of normal dart gland tubules stained with PAS showing peripheral cells (PGC) and lumens (L) with luminal contents (LC). X 100.

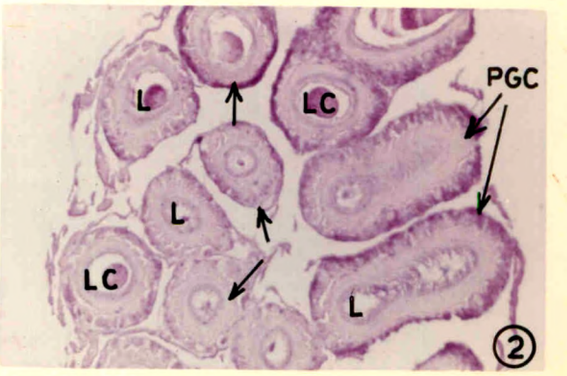
Fig. 6 : T.S. of dart gland tubules from slug injected with cerebral ganglia extract, staining with PAS and taken on 25th day after treatment, showing enhanced staining in cells (PGC) and lumens (L). X 100.

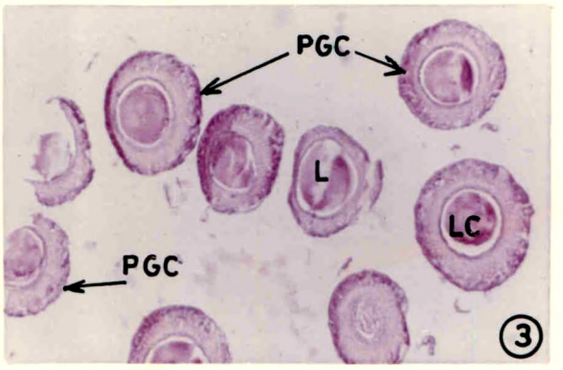
Fig. 7 : T.S. of normal dart gland tubules stained with PAS showing staining in luminal contents (LC) in lumens (L) and peripheral cells (PGC). X 100.

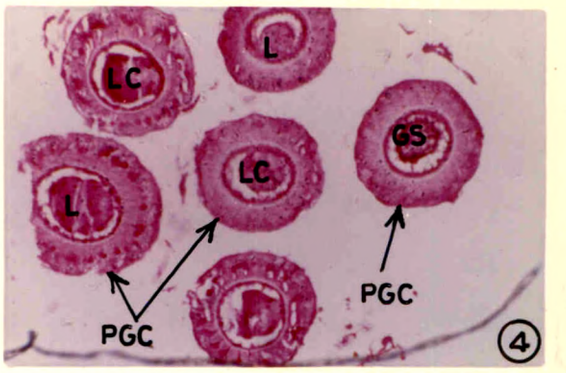
Fig. 8 : T.S. of dart gland tubules stained with PAS showing increased mucosubstances in the cells (PGC), amorphous secretion (AS) and granular droplets (GD) in slugs injected with the ovotestis extract, taken on 25th day after injections. X 640.

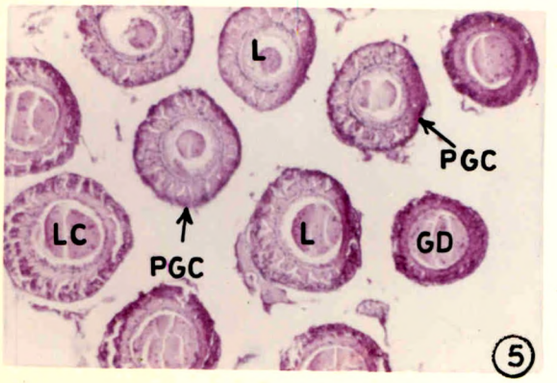
PLATE No. 4

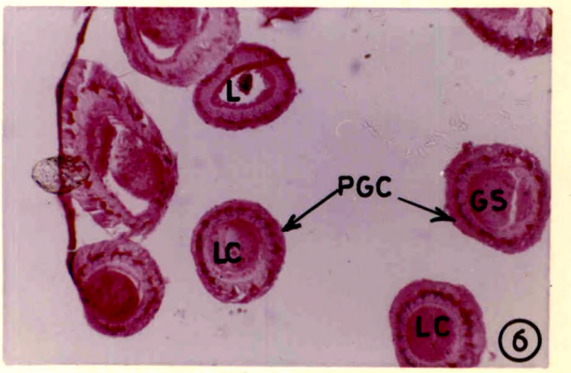

PGC
L
PGC
L
LC
LC
PGC
1

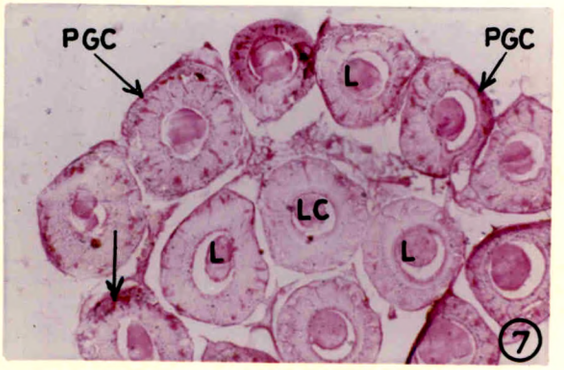

L
LC
PGC
L
LC
L
2

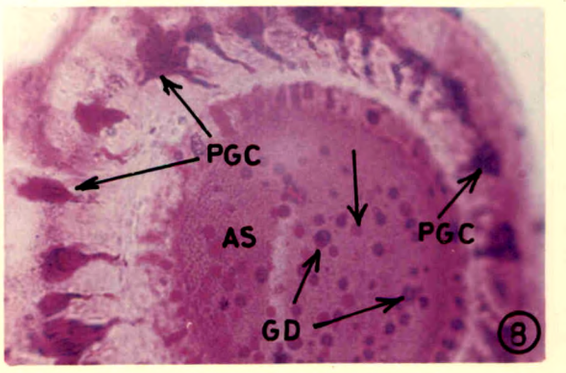

PGC
L
LC
PGC
3


LC
L
GS
LC
L
PGC
PGC
4


L
PGC
LC
PGC
L
GD
5


L
PGC
GS
LC
LC
6


PGC
PGC
L
LC
L
L
7


PGC
AS
PGC
GD
8

of blue colouration. These reactions indicated the presence of only neutral mucopolysaccharides in these cells. At a comparative level numerically they were more.

Acidic mucopolysaccharides secreting cells :

These cells showed PAS reactivity resistant to saliva digestion. They exhibited intense alcianophilia with AB both at pH 1 and 2.5. In the combined AB (pH 1, 2.5)-PAS sequential staining procedures, these cells exhibited only blue staining, there being no tings of pink staining. These reactions indicated absence of neutral mucopoly-saccharides in these cells but consisted of sulfated acidic mucopoly-saccharides.

Mixed mucopolysachharides secreting cells :

These cells exhibited an intense PAS reactivity, which was completely resistant to saliva digestion. In combined sequential AB (pH 1, 2.5)-PAS staining techniques, they showed bluish-pink staining. They exhibited alcianophilia both at AB pH 1 and pH 2.5. Thus, they showed the presence of neutral and acidic mucopolysaccharides in the combined state in these cells.

Muscular coat :

The muscle cells showed PAS reactivity which was abolished by prior saliva digestion. These cells reacted negatively with the rest

of the histochemical staining procedures. These results indicated the presence of glycogen in these cells.

Luminal contents :

The amorphous watery secretion showed histochemical reactivities similar to neutral mucopolysaccharides secreting cells, described above, indicating the presence of only neutral mucins in them probably elaborated and secreted by these cells.

As against this, the granules and droplets embeded in the watery secretion showed presence of neutral, acidic and mixed mucopolysaccharide contents in them, probably secreted by the respective peripheral glandular cells of this gland.

B) Effects of optic tentacular neurohormones on the elaboration of mucopolysaccharides by dart gland :

The optic tentacles neurohormones altered the capacity of elaboration of mucosubstances in the various cells and tissues of the dart gland. The histochemical data on the distribution and alterations are recorded in Table No.5 and also illustrated photomicrographically in Plate No.4, Figs.2 and 4 and Plate No.5, Figs.2 and 4.

When optic tentacles were ablated the concentrations of neutral, acidic and mixed mucopolysaccharides showed minimum levels and staining intensity in the various cells of the dart gland

Table No.5 : Histochemical staining reactions of **dart gland** in S. maculata under the influence of various hormones of optic tentacles, cerebral ganglia and ovotestis.

Sr. No.	Histochemical Techniques	Tissues of dart gland	Control Group	Experimental conditions						
				OTAB	OTAB + OTEI	OTIN + OTEI	OTAB+ CGEI	OTIN + CGEI	OTAB + OVEI	OTIN + OVEI
1.	PAS	NGC	+++P	+P	+++P	++++P	+++P	++++P	+++P	++++P
		AGC	+++P	++P	+++P	++++P	+++P	++++P	+++P	++++P
		MGC	++P	-	++P	+++P	++P	+++P	++P	+++P
		MB	+++P	+P	+++P	++++P	+++P	++++P	+++P	++++P
2.	Saliva diges-tion - PAS	NGC	+++P	+P	+++P	++++P	+++P	++++P	+++P	++++P
		AGC	+++P	++P	+++P	++++P	+++p	++++P	+++P	++++P
		MGC	++P	-	++P	+++P	++P	+++P	++P	+++P
		MB	-	-	-	-	-	-	-	-
3.	AB pH 1	NGC	-	-	-	-	-	-	-	-
		AGC	+++B	+B	+++B	++++B	+++B	++++B	+++B	++++B
		MGC	++B	-	+++B	++++B	+++B	++++B	+++B	++++B
		MB	-	-	-	-	-	-	-	-
4.	AB pH 2.5	NGC	-	-	+B	+B	+B	+B	+B	+B
		AGC	+++B	+B	+++B	++++B	+++B	++++B	+++B	++++B
		MGC	++B	-	++B	+++B	++B	+++B	++B	+++B
		MB	-	-	-	-	-	-	-	-
5.	AB pH 1-PAS	NGC	+++P	+P	+++P	++++P	+++P	++++P	+++P	++++P
		AGC	+++B	++B	+++B	++++B	+++B	++++B	+++B	++++B
		MGC	++BP	-	++BP	+++BP	++BP	+++BP	++BP	++++BP
		MB	+++P	+P	+++P	++++P	+++P	++++P	+++P	++++P
6.	AB pH 2.5-PAS	NGC	+++P	+P	+++P	++++P	+++P	++++P	+++P	++++P
		AGC	+++B	++B	+++B	++++B	+++B	++++B	+++B	++++B
		MGC	++BP	-	+++BP	++++BP	+++BP	++++BP	+++BP	++++BP
		MB	+++P	+P	+++P	++++P	+++P	++++P	+++P	++++P

N.B.: The nature, histochemical reactivities and variations of the mucosubstances in the amorphous secretory and secretory droplets in the luminal contents were similar to the corresponding mucosubstances in the peripheral glandular cells of the dart gland.

Captions to Figures

PLATE NO. 5

(Histochemical alterations in the mucosubstances of dart gland in S. maculata under the effects of hormones)

Fig. 1 : T.S. of normal dart gland tubules stained with AB pH 2.5. Note some of the tubules with empty lumens (L). X 100.

Fig. 2 : T.S. of dart gland tubules of the slugs with ablated optic tentacles stained with AB pH 2.5, showing reduction in staining in lumens (L) and cells. X 100.

Fig. 3 : T.S. of normal dart gland tubules stained with AB pH 2.5, showing secretory peripheral cells (PGC) and lumens (L). X 100.

Fig. 4 : T.S. of dart gland tubules stained with AB pH 2.5 of slugs with intact tentacles and injected with optic tentacles extract, showing increased secretion in lumens (L) and cells (GC). X 100.

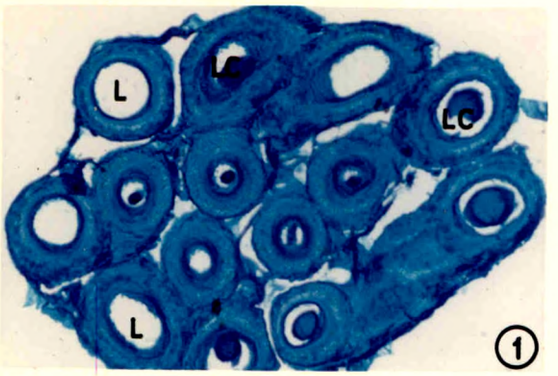
Fig. 5 : T.S. of normal dart gland tubules stained with AB pH 2.5, showing alcianophilic cells (PGC) and luminal content (LC). X 100.

Fig. 6 : T.S. of dart gland tubules of slug with ablated optic tentacles and injected with crebral ganglia extract. Note slight increase in secretory cells (PGC) mucosubstances and in the luminal contents (LC) in lumens (L). X 100.

Fig. 7 : T.S. of normal dart gland tubules stained with AB pH 2.5 showing normal secretion in cells (PGC) and in luminal (L) granules (GS). X 100.

Fig. 8 : T.S. of dart gland tubules stained with AB pH 2.5 of slug with ablated optic tentacles and injected with ovotestis extract, showing increase in staining in cells (PGC) and in luminal contents (LC). X 100.

PLATE No. 5



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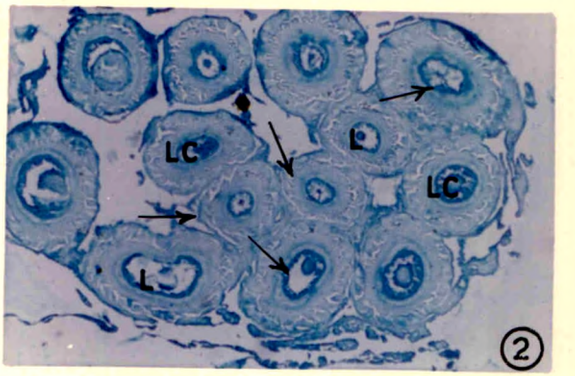

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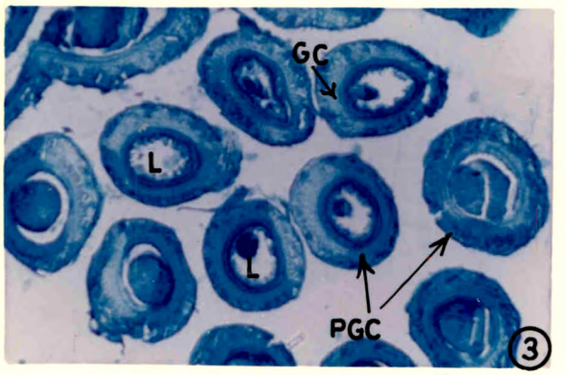

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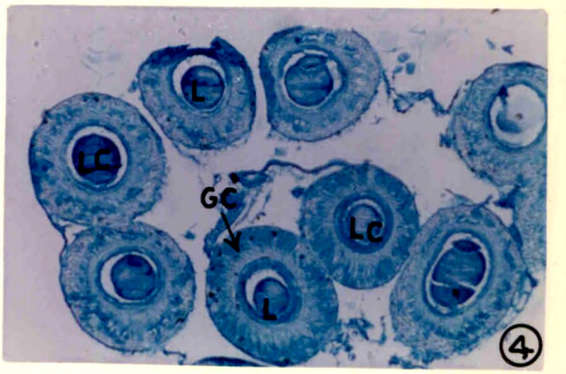

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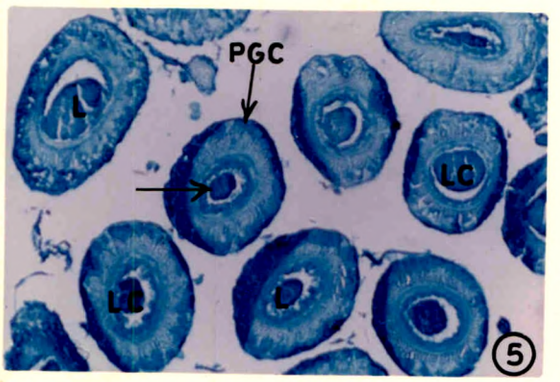

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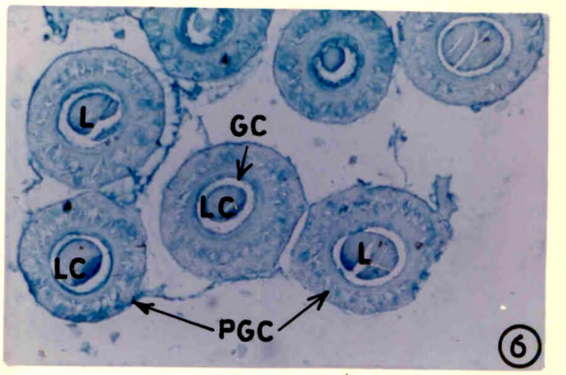

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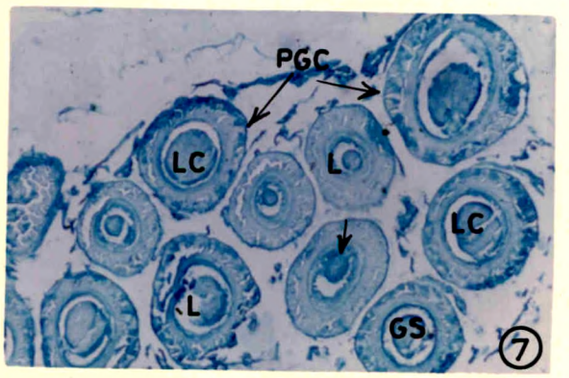

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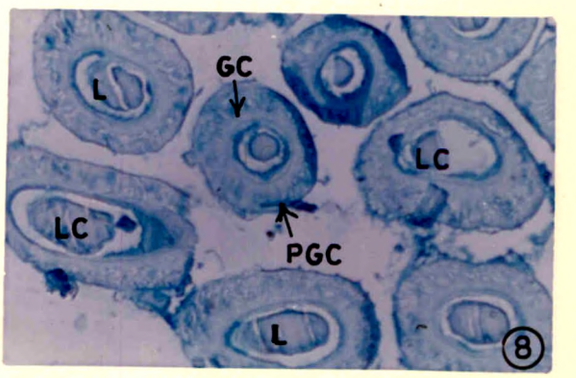


8

(Plate No.4, Fig.2). Some of the lumens of the tubules were empty and their contents showed very faint staining (Plate No.4, Fig.2). The luminal droplets were not visible.

On the other hand, when the extracts of optic tentacular neurohormones were injected to the slugs the elaboration of various mucopolysaccharides in the glandular cells were increased. Their staining intensities were enhanced (Plate No.4, Fig.4; Plate No.5, Fig.4). The concentrations, number and staining intensity of the granular secretory material in the lumens were increased (Plate No.4, Fig.4; Plate No.5, Fig.4). These effects were more pronounced in the slugs with intact optic tentacles than those of the slugs with ablated optic tentacles.

C) **Effects of cerebral ganglionic neuromones on the elaboration of mucopolysaccharides of dart gland** :

The neurohormones of the cerebral ganglia changed the mucopolysaccharides content in the various cells of the dart gland. These alterations in the elaboration of mucopolysaccharides are recorded in Table No.5 and also illustrated photomicrographically in Fig.6 of Plate Nos.2 and 3.

These effects were very much similar to those of the neurohormones of the optic tentacles. They increased synthesis, concentration and staining intensities of neutral, acidic and mixed mucopoly-saccharides in the peripheral glandular cells and in the

luminal droplets of the dart gland. As in case of optic tentacular neurohormones, the cerebral ganglionic neurohormones showed their effects more pronounced in the slugs with intact optic tentacles than those of the slugs with ablated optic tentacles.

D) Effects of ovotesticular hormones on the elaboration of mucopolysaccharides of dart gland :

The ovotesticular hormones also affected the elaboration of mucopolysaccharides in the various cells of the dart gland. The changes were more intense than those observed after the injections of neurohormones of the optic tentacles and cerebral ganglia. But they were similar to those observed after the injections of these two neurohormones. The changes showed increased concentrations of various mucosubstances increase in the size and number of mucosubstances elaborating cells and their staining intensities.

These histochemical alterations in the mucosubstances elaboration under the influence of ovotesticular hormones are recorded in Table No.5 and also illustrated photomicrographically in Plate No.4, Fig.8 and Plate No.5, Fig.8. These effects were more pronounced in the slugs with intact optic tentacles than those in the slugs with ablated optic tentacles.

iii) Biochemical Observations :

A) Biochemical observations on the glycogen, proteins and cholesterol in the normal dart gland :

The normal per cent values of glycogen, proteins and cholesterol in the dart gland of slugs of control group are recorded in Table No.6.

The concentration of glycogen, proteins and cholesterol was 7.65 mg, 5.01 mg and 6.75 mg per cent wet tissue of dart gland, respectively.

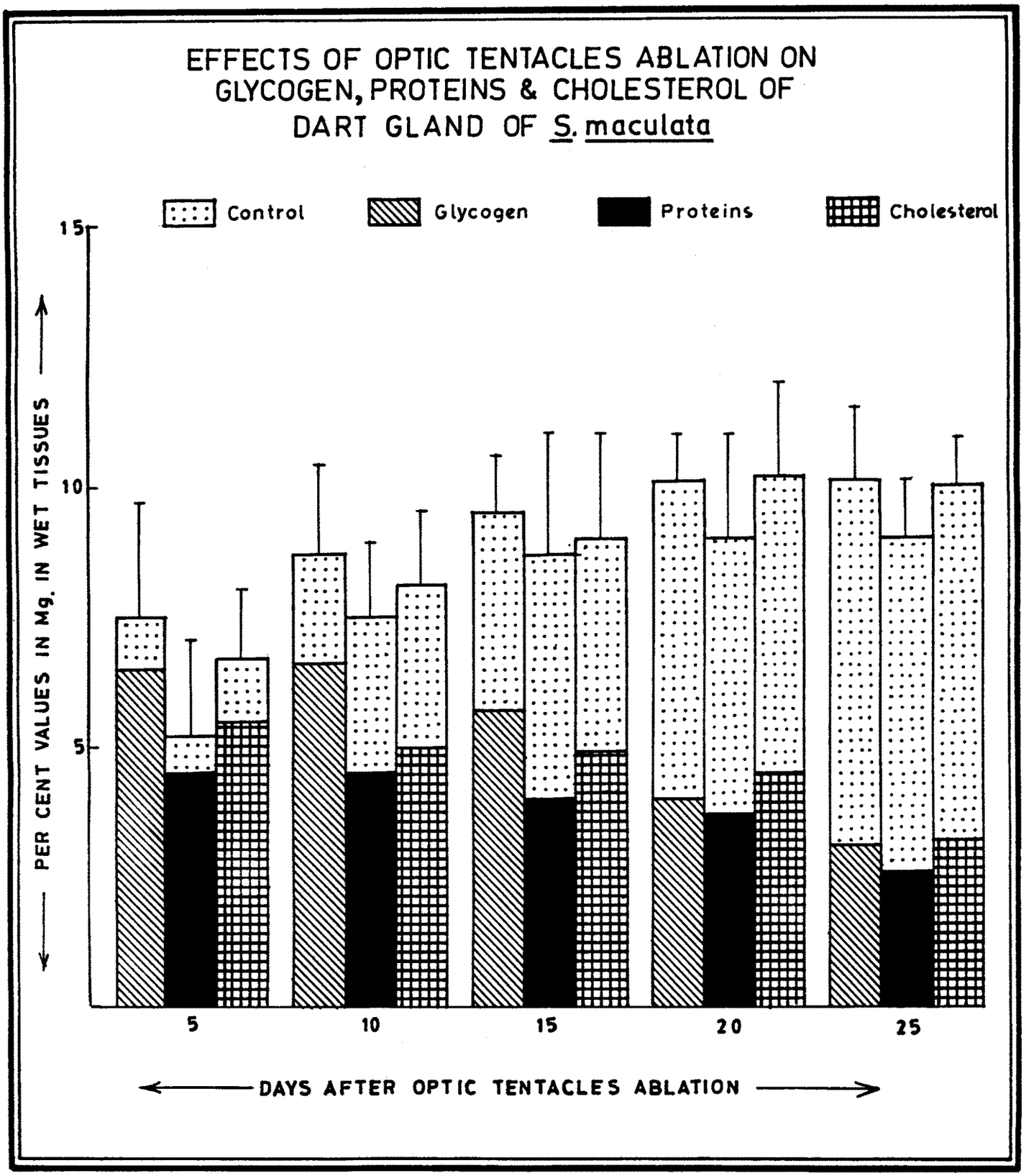
B) Effects of optic tentacular neurohormones on the glycogen, proteins and cholesterol of dart gland :

The optic tentacular neurohormones changed the concentrations of glycogen, proteins and cholesterol of the dart gland. These altered values have been recorded in Table No.6 and they are illustrated graphically in Graph Nos.5 and 6.

When the optic tentacles of the slugs were ablated the concentrations of glycogen, proteins and cholesterol were droped upto 6.50 mg%, 4.55 mg % and 5.50 mg % on the 5th day after ablation, respectively. These values further decreased through the intermediate values and reached a minimum level 3.12 mg %, 2.68 mg % and 3.20 mg % on 25th day of ablation, respectively.

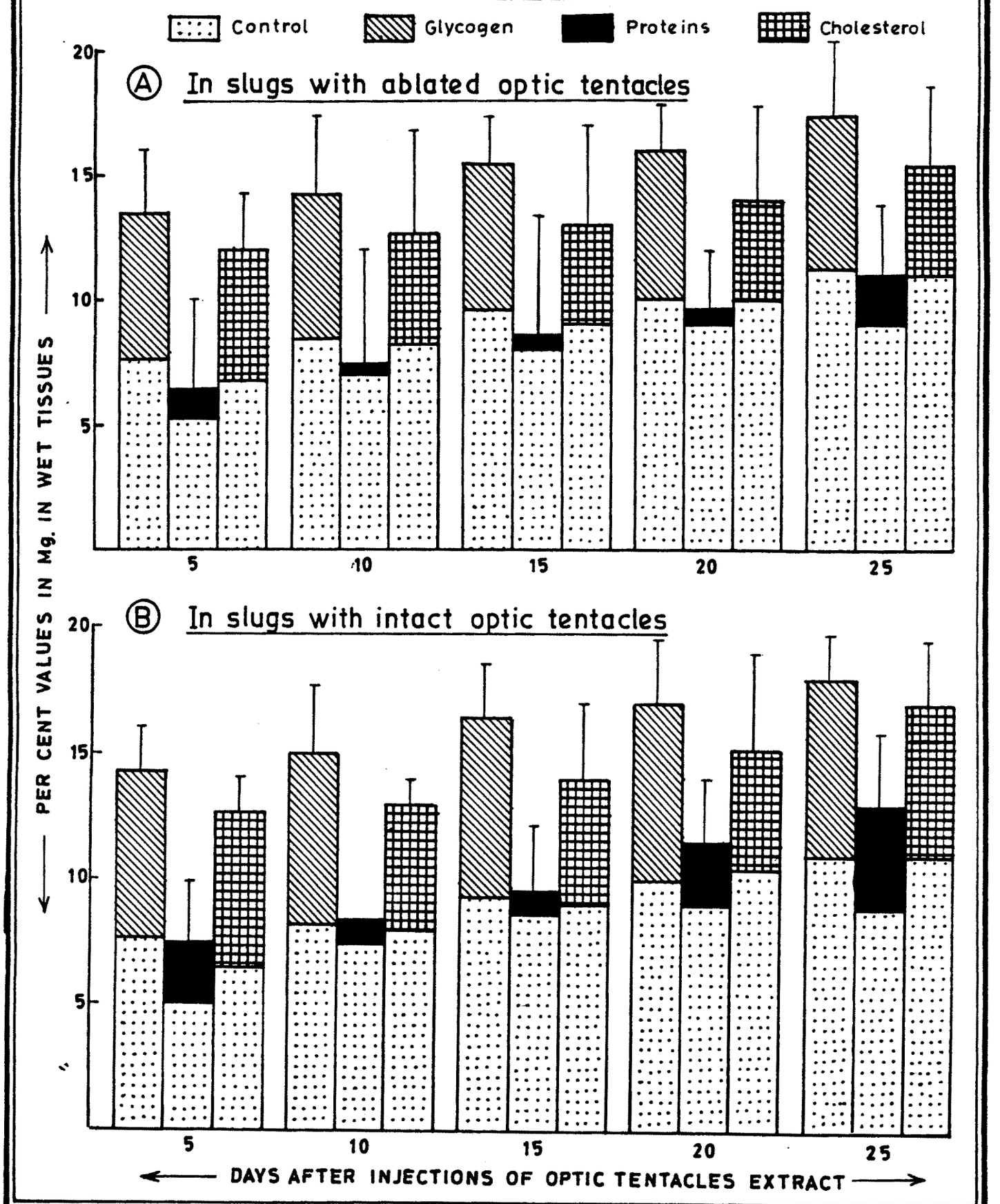
Table No.6 : Comparative data on variations in the glycogen, proteins and cholesterol in **dart gland** of S. maculata under the influence of hormones of optic tentacles, cerebral, ganglia and ovotestis.

Sr. No.	Experimental conditions	Nutrients	VALUES DURING EXPERIMENTAL DAYS 5	10	15	20	25
1	Control	Glycogen	7.65 ± 0.81	8.76 ± 0.87	9.50 ± 0.25	10.00 ± 0.26	11.15 ± 0.36
		Proteins	5.01 ± 0.02	7.50 ± 0.34	8.68 ± 0.26	9.00 ± 0.16	9.05 ± 0.37
		Cholesterol	6.75 ± 0.06	8.15 ± 0.89	9.00 ± 0.36	10.25 ± 0.15	11.00 ± 0.91
2	OTAB	Glycogen	6.50 ± 0.40	6.06 ± 0.04	5.78 ± 0.06	4.00 ± 0.01	3.12 ± 0.01
		Proteins	4.55 ± 0.35	4.50 ± 0.06	4.00 ± 0.07	3.78 ± 0.01	2.68 ± 0.01
		Cholesterol	5.50 ± 0.25	5.00 ± 0.09	4.98 ± 0.08	4.50 ± 0.02	3.20 ± 0.02
3	OTAB+OTEI	Glycogen	13.71 ± 0.81	14.50 ± 0.97	15.78 ± 0.81	16.20 ± 0.97	17.50 ± 0.84
		Proteins	6.24 ± 0.64	7.62 ± 0.50	8.25 ± 0.25	9.50 ± 0.81	11.00 ± 0.88
		Cholesterol	12.00 ± 0.50	12.98 ± 0.82	13.24 ± 0.66	14.86 ± 0.93	16.30 ± 0.92
4	OTIN+OTEI	Glycogen	14.01 ± 0.23	15.00 ± 0.67	16.57 ± 0.34	17.00 ± 0.33	18.15 ± 0.92
		Proteins	7.42 ± 0.44	8.40 ± 0.25	9.50 ± 0.23	11.50 ± 0.44	13.00 ± 0.51
		Cholesterol	12.62 ± 0.64	13.00 ± 0.46	14.00 ± 0.69	15.28 ± 0.56	17.00 ± 0.64
5	OTAB+CGEI	Glycogen	15.26 ± 0.23	15.78 ± 0.42	16.50 ± 0.68	17.78 ± 0.34	18.86 ± 0.89
		Proteins	8.01 ± 0.32	9.13 ± 0.42	10.86 ± 0.24	12.00 ± 0.89	12.78 ± 0.74
		Cholesterol	13.00 ± 0.89	13.90 ± 0.64	14.60 ± 0.02	15.78 ± 0.93	17.00 ± 0.72
6	OTIN+CGEI	Glycogen	15.58 ± 0.46	16.20 ± 0.66	17.78 ± 0.28	18.25 ± 0.98	19.00 ± 0.64
		Proteins	9.05 ± 0.66	10.14 ± 0.74	11.26 ± 0.38	12.50 ± 0.59	13.86 ± 0.22
		Cholesterol	13.07 ± 0.82	14.01 ± 0.84	15.40 ± 0.84	16.00 ± 0.69	17.25 ± 0.15
7	OTAB+OVEI	Glycogen	12.38 ± 0.46	13.46 ± 0.64	14.62 ± 0.88	16.48 ± 0.74	17.30 ± 0.11
		Proteins	6.46 ± 0.32	8.25 ± 0.99	9.29 ± 0.64	10.45 ± 0.62	11.40 ± 0.06
		Cholesterol	12.50 ± 0.94	13.02 ± 0.97	14.00 ± 0.24	14.98 ± 0.64	16.12 ± 0.07
8	OTIN+OVEI	Glycogen	12.88 ± 0.66	14.00 ± 0.21	15.52 ± 0.33	17.00 ± 0.72	18.50 ± 0.21
		Proteins	7.00 ± 0.32	8.10 ± 0.04	10.35 ± 0.21	12.00 ± 0.23	13.00 ± 0.13
		Cholesterol	13.00 ± 0.60	14.00 ± 0.94	14.78 ± 0.89	16.00 ± 0.97	17.62 ± 0.94


EFFECTS OF OPTIC TENTACLES ABLATION ON GLYCOGEN, PROTEINS & CHOLESTEROL OF DART GLAND OF S. maculata
Control
Glycogen
Proteins
Cholesteral
15
10
5
PER CENT VALUES IN Mg. IN WET TISSUES
5
10
15
20
25
DAYS AFTER OPTIC TENTACLES ABLATION

GRAPH No. 5

EFFECTS OF NEUROHORMONES IN OPTIC TENTACLES ON DART GLAND GLYCOGEN, PROTEINS & CHOLESTEROL OF S. maculata



GRAPH No.6

On the other hand the concentrations of all three nutrients were increased after injections of extract of optic tentacles. In the slugs with ablated optic tentacles, these values of glycogen, proteins and cholesterol were reached upto 17.50 mg%, 11 mg% and 16.30 mg% on the 25th day after the injections, respectively. But these values were still higher in the slugs with intact optic tentacles showing 18.15 mg%, 13 mg% and 17 mg% concentrations of these three nutrients on the 25th day after the injections, respectively.

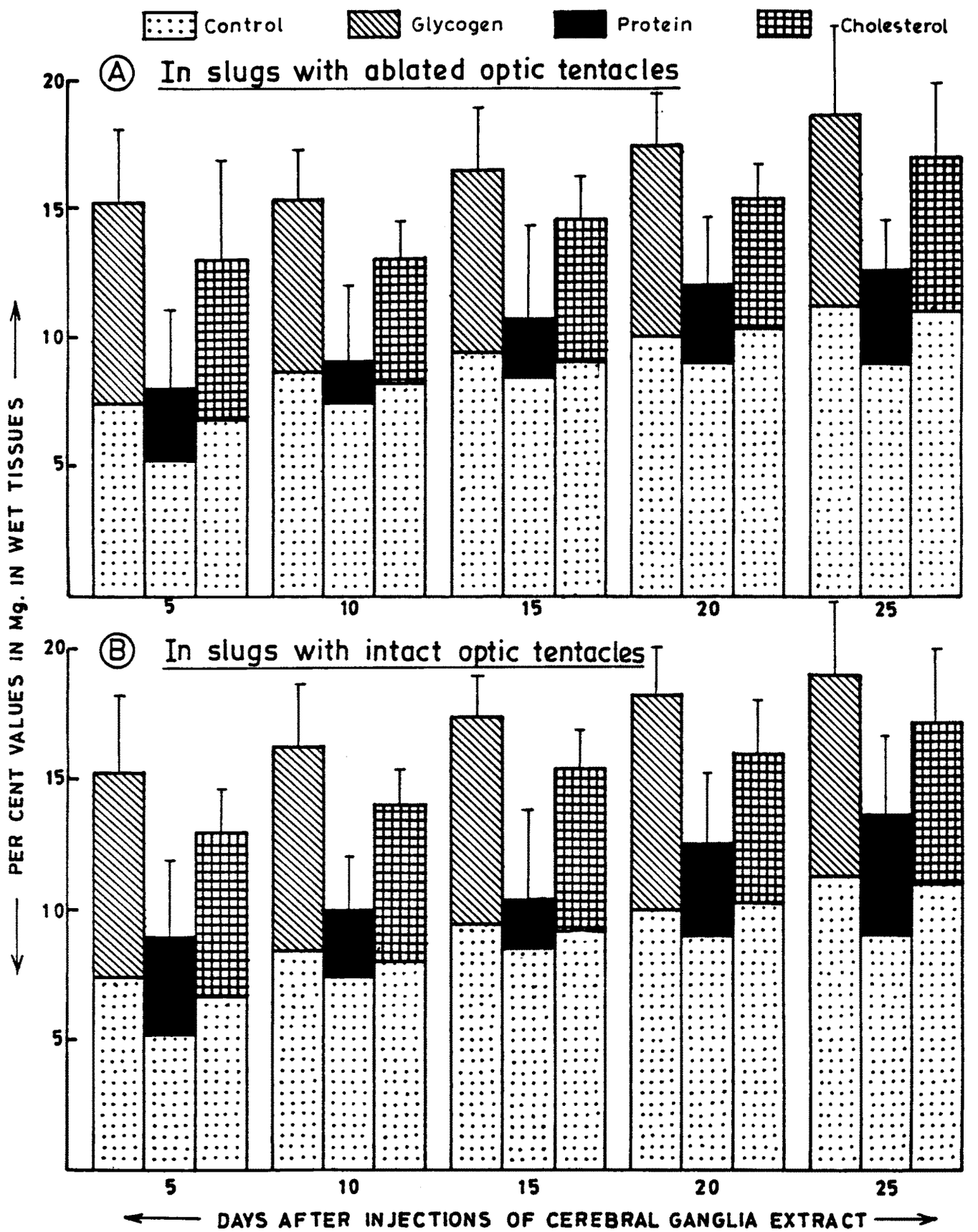
Thus, the results indicated that the withdrawl of the neurohormones by ablation of tentacles decreased the concentrations of glycogen, proteins and cholesterol in the dart gland. But addition of optic tentacular neurohormones by injecting their extracts increased the concentrations of these nutrients in this gland.

C) **Effects of cerebral ganglionic neurohormones on the glycogen, proteins and cholesterol of dart gland** :

The injections of the extract of the cerebral ganglia also altered the concentrations of glycogen, proteins and cholesterol of the dart gland. These changes are recorded in Table No.6 and shown graphically in Graph No.7.

The injection of cerebral ganglionic neurohormones to the slugs with ablated optic tentacles increased the concentrations of glycogen, proteins and cholesterol upto 18.86 mg%, 12.78 mg% and

EFFECTS OF NEUROHORMONES IN CEREBRAL GANGLIA ON DART GLAND GLYCOGEN, PROTEINS & CHOLESTEROL OF S. maculata



GRAPH No. 7

19 mg% on 25th day after injections, respectively, through the intermediate values from the 5th day to the 20th day.

Such injections to the slugs with intact optic tentacles showed more increase upto 19 mg% for glycogen, 13.86 mg% for proteins and 17.25 mg% for cholesterol on the 25th day after injections.

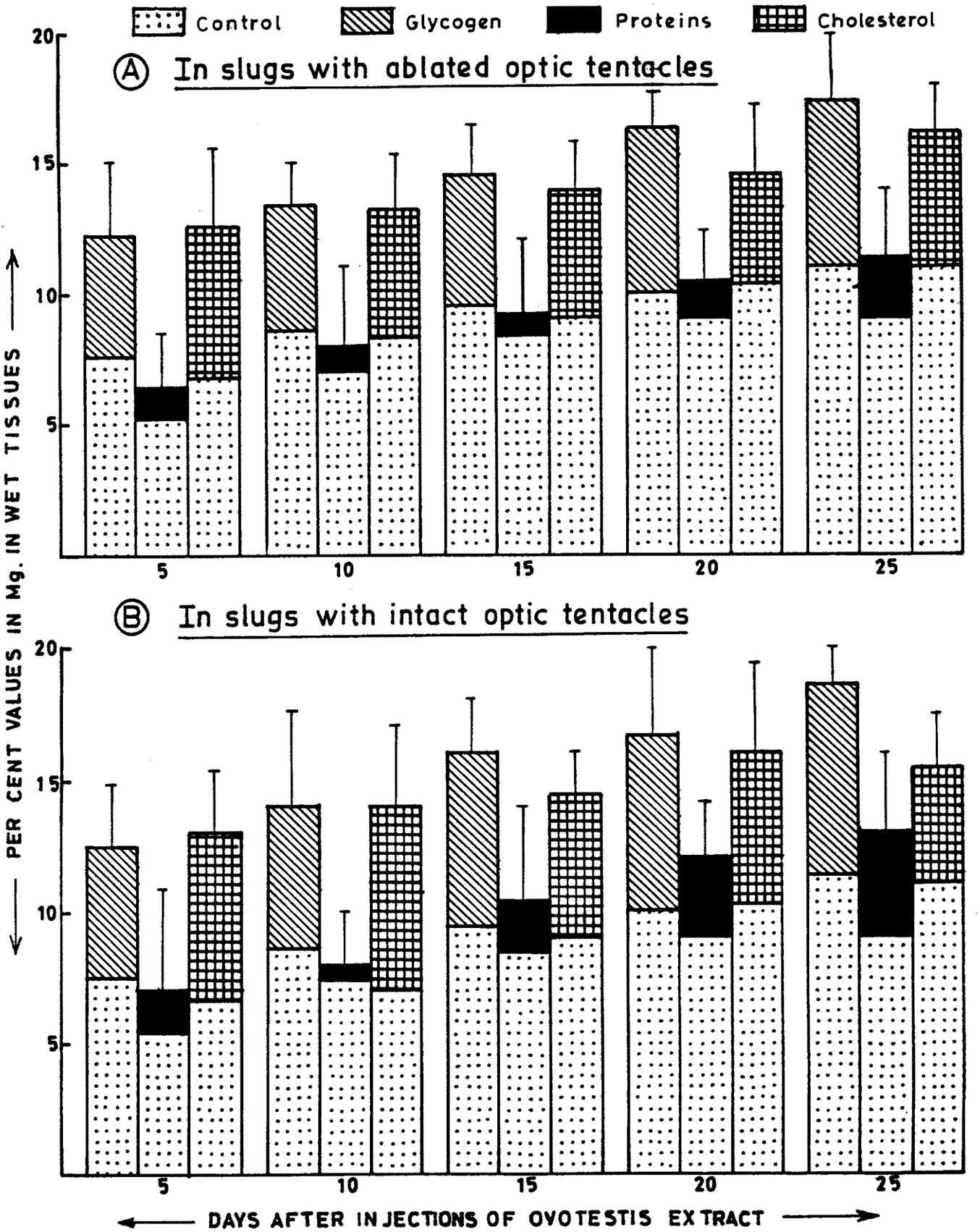
This indicated the stimulus for synthesis and storage for glycogen, proteins and cholesterol from the neurohormones of the cerebral ganglia in the dart gland.

D) Effects of ovotesticular hormones on the glycogen, proteins and cholesterol of dart gland :

The hormones of the ovotestis also increased the concentrations of glycogen, proteins and cholesterol in the dart gland. The alterations are recorded in Table No.6 and they are shown graphically in Graph No.8.

Mg per cent concentration of glycogen in the slugs with ablated optic tentacles was 12.38 on 5th day which increased upto 17.30 on 25th day after injections of ovotestis extracts. Mg per cent concentration of proteins was 6.46 on the 5th day which increased upto 11.40 on 25th day after injection of ovotestis extracts and mg per cent concen-tration of cholesterol was 12.50 on 5th day increased upto 16.12 on 25th day after injections.

EFFECTS OF HORMONES IN OVOTESTIS ON DART GLAND GLYCOGEN, PROTEINS & CHOLESTEROL OF S. maculata



GRAPH No. 8

These values of glycogen, proteins and cholesterol in the slugs with intact optic tentacles were 12.88 mg% and 18.50 mg%, 7 mg% and 13 mg%, and 13 mg% and 17.62 mg% on the 5th and 25th day, respectively, after the injections of ovotestis extracts.

These results indicated that the hormones of the ovotestis stimulated the elaboration and storage of glycogen, proteins and cholesterol in the various cells of the dart gland of S. maculata.

3. PENIS

i) Histological Observations :

A) Histological observations on Normal Penis :

The histological structure of the penis and the penis sheath of S. maculata visualised in the H-E staining technique consisted of the outer-most surface of the penis which was chitinised and covered with cuboidal epithelium. Several unicellular glands opened on the surface. These glands contained secretory material packed in them, which stained pink in H-E technique. On the outer surface of the proximal half of the penis, conical squarish outgrowths or papillae were observed, which believed to perform the irritating function and served to give a grip at the time of copulation. Outer epithelium was followed by a thin strip of connective tissue, which stained pink in H-E staining technique. The connective tissue layer was followed by a longitudinal muscles in which transverse and oblique strands of muscle fibers were scattered. They stained pink

to red in H-E technique. The rest of the interior of the penis consisted of loose connective tissue with large blood spaces. Interlocking muscle bands occured in the loose tissue. The loose tissue in the posterior half of the penis was traversed by small duct of vas deferens running obliquely through the penis.

The Penis sheath covered the penis externally. It consisted of a thin connective tissue band at the outer surface, followed by muscular coat of longitudinal bundles with scattered circular muscle fibers. Next to this was thin connective tissue covered by epithelial lining. The connective tissue contained many multicellular glands embeded in it, which stained blue in H-E technique.

B) Effects of optic tentacular neurohormones on the histology of penis :

The neurohormones elaborated by the optic tentacles changed the histological characters of the penis. The changes were related to the size of the penis and consequently the size of its tissues, to the staining intensity of the various tissues, to the number of unicellular and multicellular glands in the penis and penial sheath, respectively, and to the concentration of secretion in the various glandular cells.

In general, when the optic tentacular hormones were stoped by their ablation, the size of the penis and its tissues was very much reduced. The histological staining intensities of various tissues were reduced. The numbers of unicellular glands in the outermost layer

of penis and the multicellular glands in the innermost layers of penis sheath was decreased considerably and their secretion concentration remained in the traces.

All these changes were reversed after the injections of extract of the optic tentacles to the slugs even with their optic tentacles were ablated. But such changes were distinct in the slugs with their optic tentacles intact. These results indicated the stimulatory effect of neurohormones elaborated in the optic tentacles for the cellular differentiation, growth and synthesis of secretory products in the penis and in its sheath.

C) **Effects of cerebral ganglionic neurohormones on the histology of penis :**

The neurohormones elaborated by the cerebral ganglia also altered the histology of the penis. These changes were similar to those observed under the effect of neurohormones of the optic tentacles when they were injected through their extracts. Therefore, it indicated that the neurohormones of the cerebral ganglia also stimulated the cellular differentiation, growth and synthesis of secretion in the various cells and glands in the penis and penial sheath of S. maculata.

D) Effects of ovotesticular hormones on the histology of the penis :

The ovotesticular hormones affected the histological picture of the penis. The effects were enlargement of penis, increased staining intensities of histochemical reactivities, increased number of unicellular gland of the penis and multicellular glands of the penis sheath, etc. These changes were more pronounced in the slugs with intact optic tentacles than those of the slugs with ablated optic tentacles.

ii) Histochemical observations :

A) Elaboration of mucosubstances by the normal penis and penis sheath :

The histochemical data on some important staining reactions employed in the present investigation of the penis and penis sheath are recorded in Table No.7 according to the visually estimated intensity and shade with four plus (++++) representing the strongest activity. The distribution of mucosubstances in the penis and in the penis sheath are illustrated photomicrographically in Fig.1,3,5,7 of Plate No.6 and Plate No.7. The histochemical results requiring further description and consideration are presented hereafter along with the interpretations of the histochemical staining reactions.

Table No.7 : Histochemical staining reactions of the **penis** of S. maculata

Sr. No.	Histochemical Techniques	TISSUES OF THE PENIS AND ITS SHEATH							
		Penis				Penis Sheath			
		Unicellular glands		Muscles	Connect-	Multicellular glands		Muscles	Connec-
		UG	UG	(MP)	ive tissue	(MG)	(MG)	(MS)	tive tissue
		Type I	Type II		(PCT)	Type I	Type II		(SCT)
1	PAS	++P	++P	+++P	++P	+++P	+++P	++P	+P
2	Saliva digestion - PAS	++P	++P	-	++P	+++P	+++P	-	+P
3	AB pH 1	++B	-	-	++B	+++B	-	-	+B
4	AB pH 2.5	++B	++B	-	++B	+++B	+++B	-	+B
5	AB pH 1 - PAS	++B	++P	+++P	++B	+++B	+++P	++P	+B
6	AB pH 2.5 - PAS	++B	++B	+++P	++B	+++B	+++B	++P	+B

Captions to Figures

PLATE NO. 6

(Histochemical alterations in the mucosubstances of Penis in S. maculata under the effects of hormones)

Fig. 1 : T.S. of normal penis (P), stained with AB (pH 2.5) - PAS, showing vas deferens (VS) in the center, muscles (MT), loose connective tissue (LT) and penial sheath. X 100.

Fig. 2 : T.S. of penis (P) of slug with ablated optic tentacles, stained with PAS showing decreased staining in unicellular glands (UG), penis sheath (PS), muscles (MT) and connectie tisue (LT). Vas deferens (VS) is at the centre. X 100.

Fig. 3 : T.S. of normal penis (P) showing intense PAS staining at peripheryl layer (OE), muscles (MT), connective tissue (LT) and in vas deferens (VS). X 100.

Fig. 4 : T.S. of penis (P) of slug with optic tentacles, injected with optic tentacles extract showing enhanced PAS staining in muscles (MT), connective tissue (LT) and Penis sheath (PS). X 100.

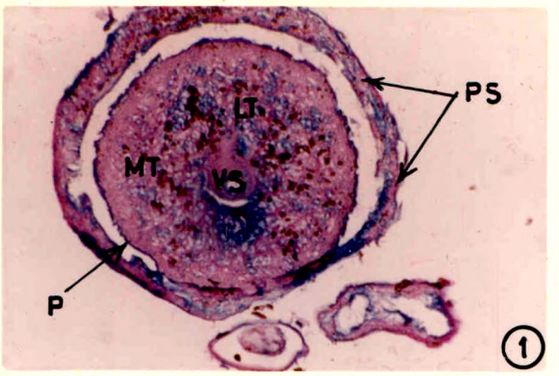
Fig. 5 : T.S. of normal Penis (P) showing vas deferens (VS), outer epithelium (OE), muscular tissue (MT) and loose connective tissue (LT). X 100.

Fig. 6 : T.S. of penis (P) of slug injected with cerebral ganglia extract, stained with AB (pH 2.5)-PAS, showing enhanced staining in multicellular gland (MG) in penis sheath (PS), in muscles (MT), connective tissue (LT), unicellular glands (UG) of penis and in vas deferens (VS). X 100.

Fig. 7 : T.S. of normal penis (P) and penis sheath (PS) stained with AB (pH 2.5)-PAS showing moderate staining in unicellular glands (UG), multicellular glands (MG), muscles (MT), connective tissue (LT) and vas deferens (VS). X 100.

Fig. 8 : T.S. of penis (P) and penis sheath (PS) from slug injected with ovotestis extract showing enhanced staining in multicellular glands (MG), unicellular glands (UG), muscles (MT) and connective tissue (LT). X 100.

PLATE No. 6



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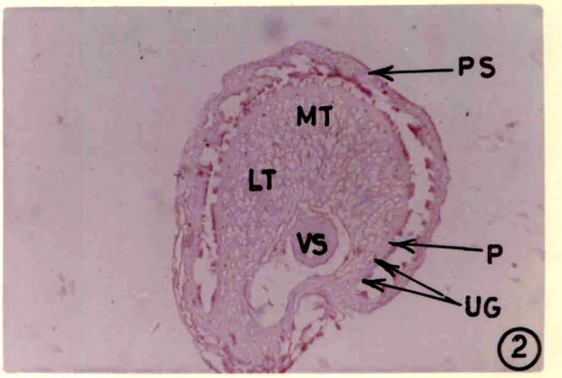

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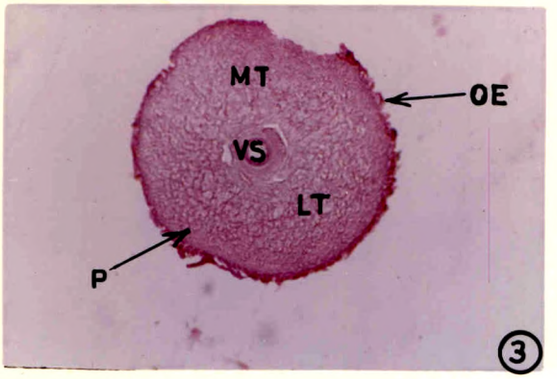

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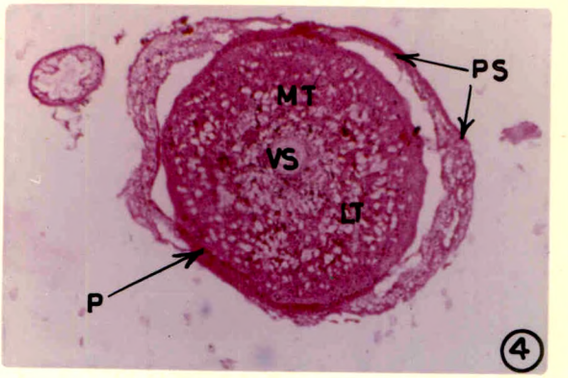

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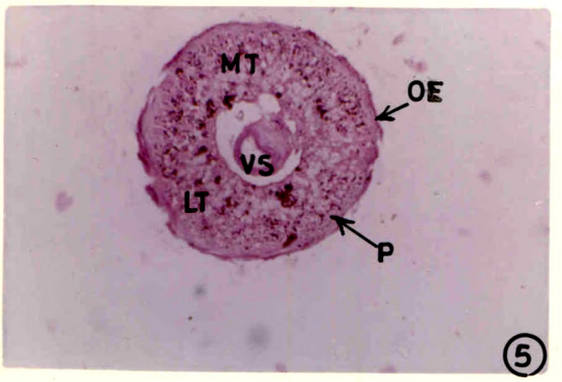

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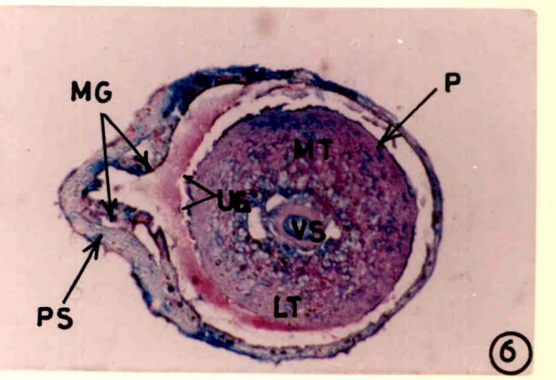

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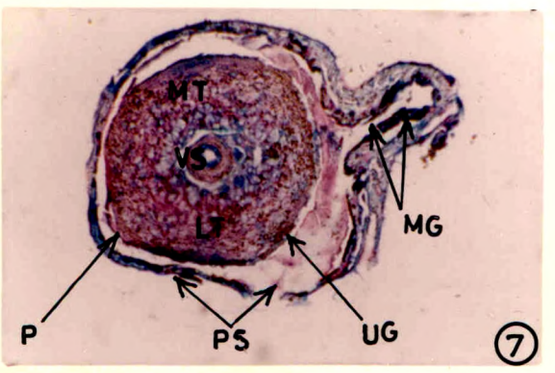

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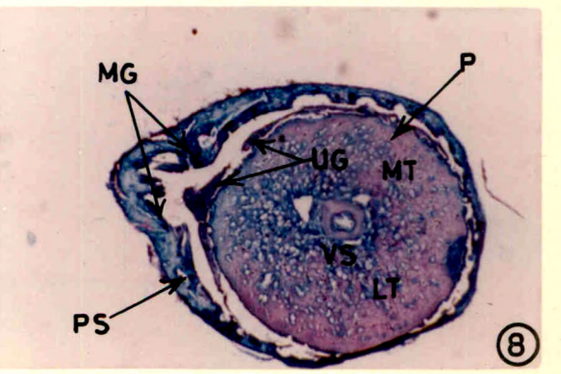

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Type 1 : Unicellular glands in penis :

The secretion of unicellular glands in penis exhibited an intense PAS reactivity, which was completely resistant to saliva digestion. They showed alcianophilia with AB both at pH 1 and 2.5. In combined sequential staining procedures AB (pH 1, 2.5)-PAS, they stained blue without pink shade of PAS. These histochemical reactions indicated the presence of the acidic mucosubstances - i.e. sulfomucins in them.

Type 2 : Unicellular glands in Penis :

The histochemical reactions indicated the presence of sialic acid in the carboxymucins (sialomucins) in the second type of unicellular glands of the penis.

Mucosubstances in the muscles and loose connective tissue of penis

The histochemical reactions indicated the presence of glycogen in the penial muscles and the presence of sulfomucins in the loose connective tissue of the penis.

Penis sheath :

The histochemical reactivities of penis sheath indicated the presence sulfomucins in the type 1 multicellular glands, sialomucins in the type 2 multicellular glands, glycogen in the muscles and weakly sulfomucins intheir connective tissue.

Captions to Figures

PLATE NO. 7

(Histochemical alterations in the mucosubstances of penis in S. maculata under the effects of hormones)

Fig. 1 : T.s. of normal penis (P) stained with AB pH 2.5, showing penis sheath (PS), multicellular glands (MG) and vas deferens (VS). X 100.

Fig. 2 : T.S. of penis (P) of the slugs with ablated optic tentacles stained with AB pH 2.5 showing reduction in staining in sheath (PS), outer epithelium (OE), muscles (MT), connective tissue (LT) and vas deferens (VS). X 100.

Fig. 3 : T.S. of normal penis (P) stained with AB pH 2.5, showing outer epithelium (OE), muscles (MT), connective tissue (LT) and vas deferens (VS). X 100.

Fig. 4 : T.S. of penis (P) stained with AB pH 2.5 of slugs with intact tentacles and injected with optic tentacles extract, showing enhanced staining in outer epithelium (OE), muscles (MT), connective tissue (LT) and in vas deferens (VS). Note blood space (BS). X 100.

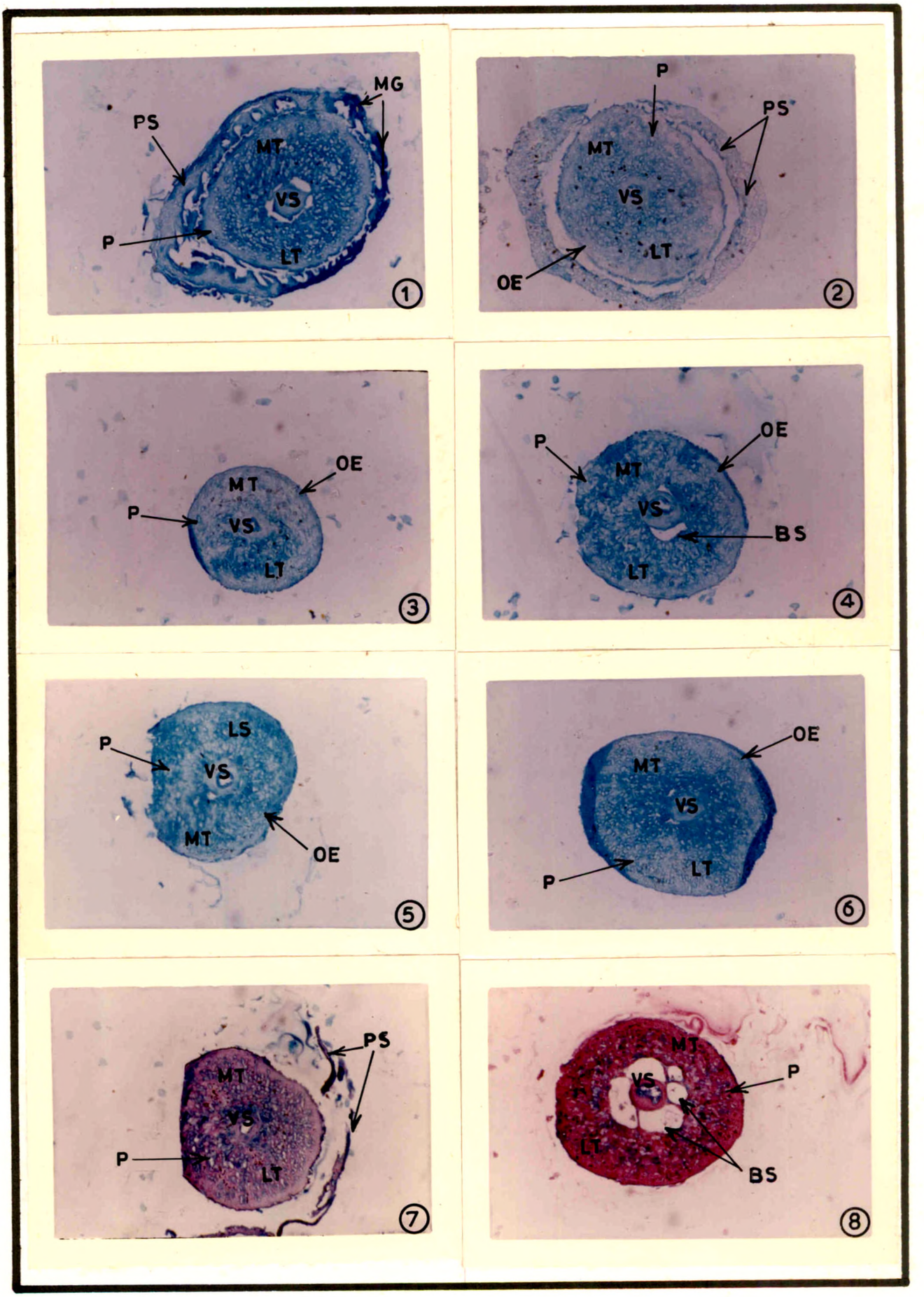
Fig. 5 : T.S. of normal penis (P) tubules stained with AB pH 2.5 showing outter epithelium (OE) muscles (MT), connective tissue (LT) and vas deferens (VS). X 100.

Fig. 6 : T.S. of penis (P) of slug with ablated optic tentacles and injected with cerebral ganglia extract, stained with AB (pH 2.5) showing enhanced staining in outer epithelium (OE), muscles (MT), connective tissue (LT) and vas deferens (VS). X 100.

Fig. 7 : T.S. of normal penis (P) stained with AB pH 2.5-PAS showing penis sheath (PS), muscles (MT), connective tissue (LT) and vas deferens (VS).

Fig. 8 : T.S. of penis (P) stained with AB pH 2.5-PAS of slug with ablated optic tentacles and injected with ovotestis extract. Note intense staining in muscles (MT), connective tissue (LT) and in vas deferens (BS). Note large blood spaces near the center (VS). X 100.

PLATE No. 7


MG
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B) Effects of optic tentacular neurohormones on the elaboration of mucosubstances by the penis and penis sheath :

The neurohormones elaborated by the optic tentacles altered the capacity of elaboration of mucosubstances by the penis and penis sheath. The histochemical data on the distribution and alterations are recorded in Table No.8 and also illustrated photomicrographically in Figs. 2 and 4 of Plate Nos.6 and 7.

When the optic tentacles were ablated the glycogen in the muscles of penis and penial sheath was totally lost, sulfomucins in the type 1 unicellular and multicellular glands were reduced, sialomucins in the type 2 unicellular and multicellular glands were diminished. The reactivities towards all the histochemical staining techniques were decreased (Plate No.6, Fig.2; Plate No.7, Fig.2).

But when the extracts of optic tentacles were injected the elaboration of all these mucosubstances were enhanced and the results were just opposite to those of the slugs with ablated optic tentacles. They showed enhanced staining reactivities (Plate No.6, Fig.4; Plate No.7, Fig.4). The concentrations of all these mucosubstances were increased and number of mucosubstances secreting cells were increased. These effects were more in the slugs with intact optic tentacles than those in the slugs with ablated optic tentacles.

Table No.8 : Histochemical staining reactions of **penis** in S. maculata under the influence of various hormones of optic tentacles, cerebral ganglia and ovotestis.

Sr. No.	Histochemical Techniques	Tissues of Penis	Control Group	Experimental conditions						
				OTAB	OTAB + OTEI	OTIN + OTEI	OTAB + CGEI	OTIN + CGEI	OTAB + OVEI	OTIN + OVEI
		UG Type I	++P	+P	+++P	++++P	+++P	++++P	+++P	++++P
1.	PAS	UG Type II	++P	+P	+++P	++++P	+++P	++++P	+++P	++++P
		MP	+++P	++P	+++P	++++P	+++P	++++P	+++P	++++P
		PCT	++P	+P	+++P	++++P	+++P	++++P	+++P	++++P
	Saliva diges-	UG Type I	++P	+P	+++P	++++P	+++P	++++P	+++P	++++P
2.	tion - PAS	UG Type II	++P	+P	+++P	++++P	+++p	++++P	+++P	++++P
		MP	-	-	-	-	-	-	-	-
		PCT	++P	+P	+++P	++++P	+++P	++++P	+++P	++++P
		UG Type I	++B	+B	+++B	++++B	+++B	++++B	+++B	++++B
3.	AB pH 1	UG Type II	-	-	-	-	-	-	-	-
		MP	-	-	-	-	-	-	-	-
		PCT	++B	+B	+++B	++++B	+++B	++++B	+++B	++++B
		UG Type I	++B	+B	+++B	++++B	+++B	++++B	+++B	++++B
4.	AB pH 2.5	UG Type II	++B	+B	+++B	++++B	+++B	++++B	+++B	++++B
		MP	-	-	-	-	-	-	-	-
		PCT	++B	+B	+++B	++++B	+++B	++++B	+++B	++++B
		UG Type I	++B	+B	+++B	++++B	+++B	++++B	+++B	++++B
5.	AB pH 1-PAS	UG Type II	++P	+P	+++P	++++P	+++P	++++B	+++P	++++B
		MP	+++P	++P	++++P	++++P	++++P	++++B	++++P	++++B
		PCT	++B	+B	+++B	++++B	+++B	++++B	+++B	++++B
		UG Type I	++B	+B	+++B	++++B	+++B	++++B	+++B	++++B
		UG Type II	++B	+B	+++B	++++B	+++B	++++B	+++B	++++B
6.	AB pH 2.5-PAS	MP	+++P	++P	++++P	++++P	++++P	++++P	++++P	++++P
		PCT	++B	+B	+++B	++++B	+++B	++++B	+++B	++++B

N.B.: Multicellular glands (Type I and Type II), muscles, connective tissue of penis sheath show identical changes in their reactivities in various experimental conditions.

C) **Effects of cerebral ganglionic neurohormones on the elaboration of mucosubstances by the penis and penis sheath** :

The neurohormones of the cerebral ganglia increased the mucosubstances similar to that of the neurohormones of the optic tentacles after their injections. Such alterations in the elaboration of mucosubstances by the penis and penis sheat are recorded in Table No.8 and also illustrated photomicrographically in Fig.6 of Plate Nos.6 and 7. But the increase in mucosubstances after injections of cerebral ganglia extract was more in slugs with intact tentacles than that of the slugs with ablated optic tentacles.

D) **Effects of ovotesticular hormones on the elaboration of mucosubstances by the penis and penis sheath** :

The ovotesticular hormones also increased the elaboration of various mucosubstances by the penis and penis sheath. The alterations in the elaboration of mucosubstances were very much similar to those observed after injections of extracts of optic tentacles and cerebral ganglia. The increase in the mucosubstances was more in the slugs with intact optic tentacles than in the slugs with ablated optic tentacles.

These histochemical alterations in the elaboration of muco-substances under the effect of ovotesticular hormones are recorded

in Table No.8 and also illustrated photomicrographically in Plate No.6, Fig.8 and Plate No.7, Fig.8.

iii) Biochemical observations :

A) Biochemical observations on the glycogen, proteins and cholestrol in the normal penis and penis sheath :

The normal per cent values of glycogen, proteins and cholesterol in the penis of the group of control slugs are recorded in Table No.9.

The concentrtion of glycogen, proteins cholesterol was 6.65 mg, 4.01 mg and 5 mg per cent wet tissue of penis, respectively.

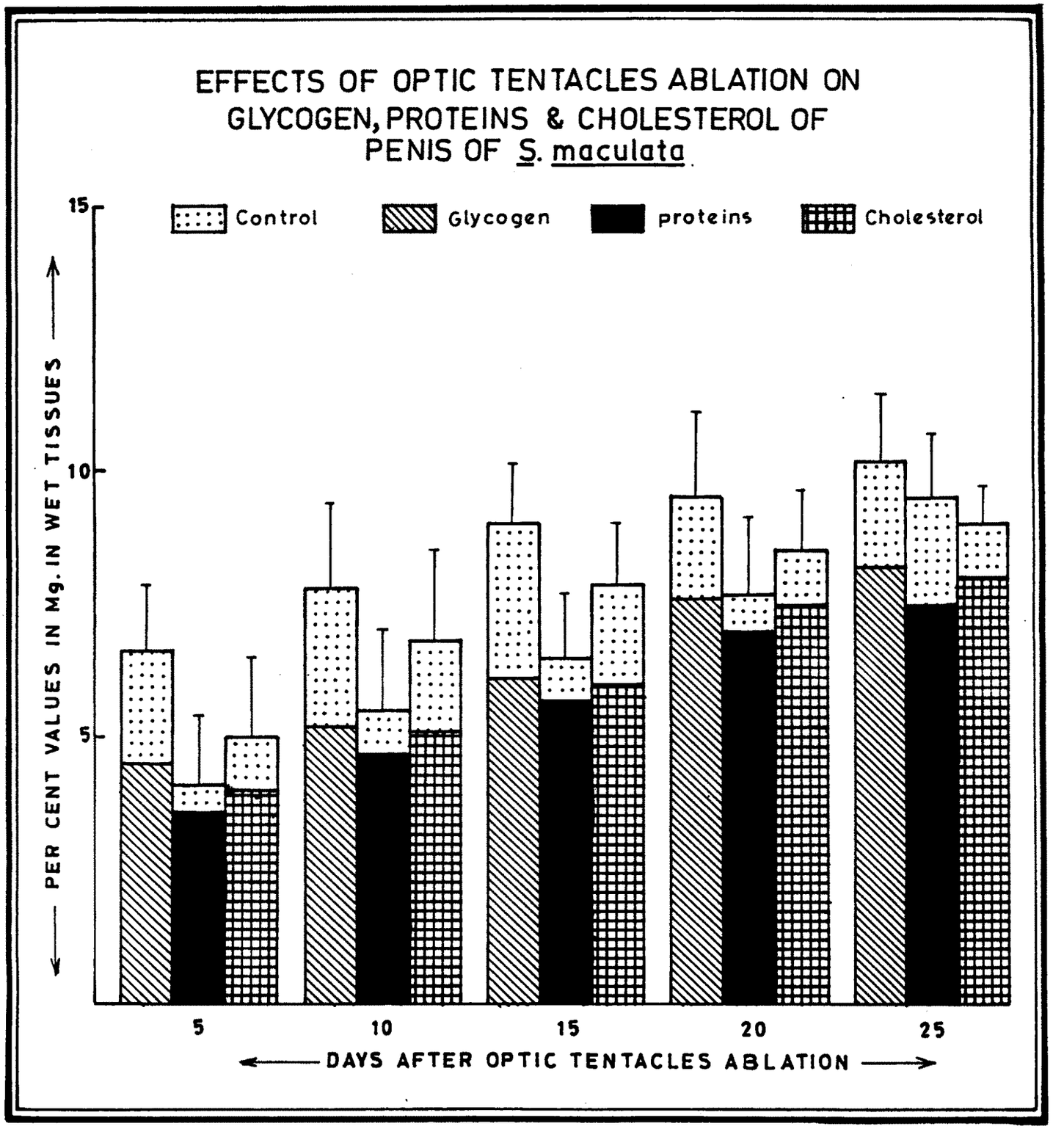
B) Effects of optic tentacular neurohormones on the glycogen, proteins and cholesterol of penis :

These neurohormones altered the concentrations of glycogen, proteins and cholesterol of the penis. Such alterations in their concentrations have been recorded in Table No.9 and they are also illustrated graphically in Graph Nos.9 and 10.

After ablation of optic tentacles the concentrations of glycogen, proteins and cholesterol were decreased from the control values to 4.5 mg%, 3.5 mg% and 4 mg% on the 5th day; 5.23 mg%, 4.75 mg% and 5.12 mg% on the 10th day; 6.15 mg%, 5.98 mg% and 6 mg% on the 15th day; 7.62 mg%, 7mg% and 7.55 mg% on

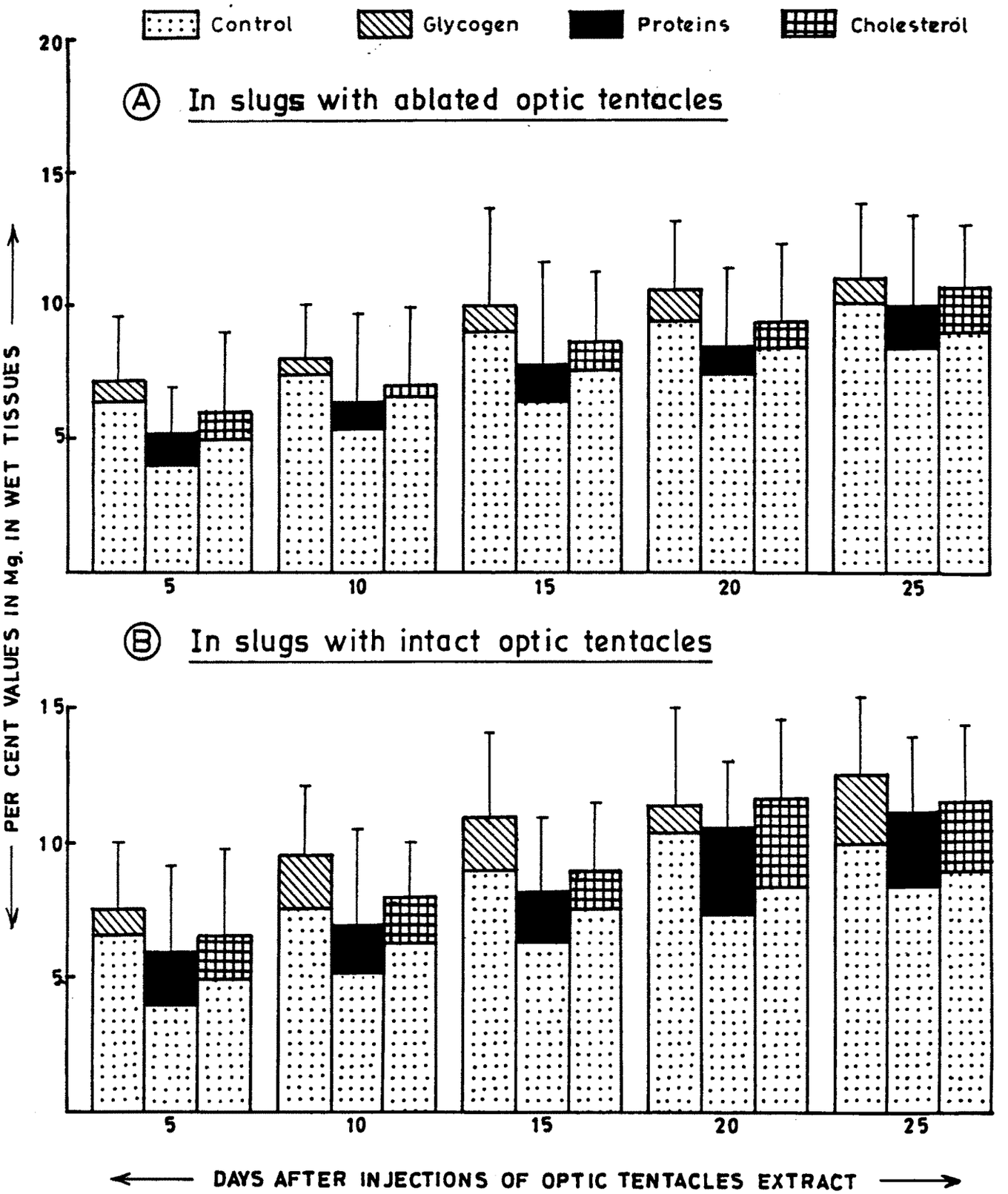
Table No. 9 : Comparative data on variations in the glycogen, proteins and cholesterol in ***penis*** of S. maculata under the influence of hormones of optic tentacles, cerebral ganglia and ovotestis.

Sr. No.	Experimental conditions	Nutrients	VALUES DURING EXPERIMENTAL DAYS 5	10	15	20	25
1	Control	Glycogen	6.65 ± 0.10	7.86 ± 0.23	9.00 ± 0.51	9.52 ± 0.13	10.20 ± 0.21
		Proteins	4.01 ± 0.20	5.53 ± 0.01	6.58 ± 0.24	7.68 ± 0.29	8.56 ± 0.36
		Cholesterol	5.00 ± 0.10	6.87 ± 0.01	7.98 ± 0.31	8.50 ± 0.62	9.00 ± 0.15
2	OTAB	Glycogen	4.50 ± 0.01	5.23 ± 0.02	6.15 ± 0.12	7.62 ± 0.43	8.21 ± 0.16
		Proteins	3.50 ± 0.01	4.76 ± 0.02	5.78 ± 0.10	7.00 ± 0.14	7.50 ± 0.12
		Cholesterol	4.00 ± 0.01	5.12 ± 0.23	6.00 ± 0.24	7.55 ± 0.26	8.06 ± 0.36
3	OTAB+OTEI	Glycogen	7.25 ± 0.10	8.05 ± 0.44	10.02 ± 0.14	10.62 ± 0.54	11.00 ± 0.39
		Proteins	5.21 ± 0.15	6.78 ± 0.49	7.83 ± 0.21	8.66 ± 0.26	10.12 ± 0.82
		Cholesterol	6.00 ± 0.16	7.33 ± 0.56	8.50 ± 0.23	9.56 ± 0.13	10.50 ± 0.78
4	OTIN+OTEI	Glycogen	7.96 ± 0.23	8.64 ± 0.67	11.00 ± 0.44	11.50 ± 0.92	12.50 ± 0.69
		Proteins	6.02 ± 0.40	7.14 ± 0.34	8.25 ± 0.76	9.00 ± 0.81	11.24 ± 0.12
		Cholesterol	6.52 ± 0.24	8.00 + 10.12	9.14 + 0.79	10.66 + 0.73	11.59 + 0.16
5	OTAB+CGEI	Glycogen	8.06 ± 0.26	9.06 ± 0.18	10.66 ± 0.32	11.86 ± 0.82	12.55 ± 0.10
		Proteins	6.25 ± 0.34	7.68 ± 0.10	8.98 ± 0.43	9.65 ± 0.21	10.25 ± 0.82
		Cholesterol	6.63 ± 0.43	8.07 ± 0.43	9.21 ± 0.61	11.00 ± 0.52	12.66 ± 0.96
6	OTIN+CGEI	Glycogen	8.76 ± 0.66	9.12 ± 0.38	11.00 ± 0.72	12.65 ± 0.33	13.76 ± 0.88
		Proteins	7.00 ± 0.21	8.05 ± 0.69	9.21 ± 0.61	11.00 ± 0.14	12.00 ± 0.76
		Cholesterol	7.60 ± 0.25	8.98 ± 0.24	9.89 ± 0.21	12.32 ± 0.34	13.03 ± 0.67
7	OTAB+OVEI	Glycogen	7.67 ± 0.69	9.07 ± 0.15	10.56 ± 0.39	11.00 ± 0.62	12.09 ± 0.21
		Proteins	6.03 ± 0.25	6.88 ± 0.54	8.00 ± 0.62	9.21 ± 0.10	10.02 ± 0.62
		Cholesterol	6.57 ± 0.25	7.00 ± 0.45	8.97 ± 0.12	10.00 ± 0.24	11.50 ± 0.42
8	OTIN+OVEI	Glycogen	8.54 ± 0.15	9.25 ± 0.37	11.00 ± 0.61	12.33 ± 0.33	13.92 ± 0.54
		Proteins	7.86 ± 0.34	8.64 ± 0.89	9.04 ± 0.50	10.00 ± 0.45	12.54 ± 0.28
		Cholesterol	8.00 ± 0.21	9.60 ± 0.92	10.00 ± 0.90	11.40 ± 0.50	13.00 ± 0.62


EFFECTS OF OPTIC TENTACLES ABLATION ON
GLYCOGEN, PROTEINS & CHOLESTEROL OF
PENIS OF S. maculata
Control
Glycogen
proteins
Cholesterol
15
10
5
PER CENT VALUES IN Mg. IN WET TISSUES
5
10
15
20
25
DAYS AFTER OPTIC TENTACLES ABLATION

GRAPH No. 9

EFFECTS OF NEUROHORMONES IN OPTIC TENTACLES ON PENIS GLYCOGEN, PROTEINS & CHOLESTEROL OF S. maculata



GRAPH No.10

the 20th day and 8.21mg%, 7.50 mg% and 8.06 mg% on the 25th day after the ablation, respectively.

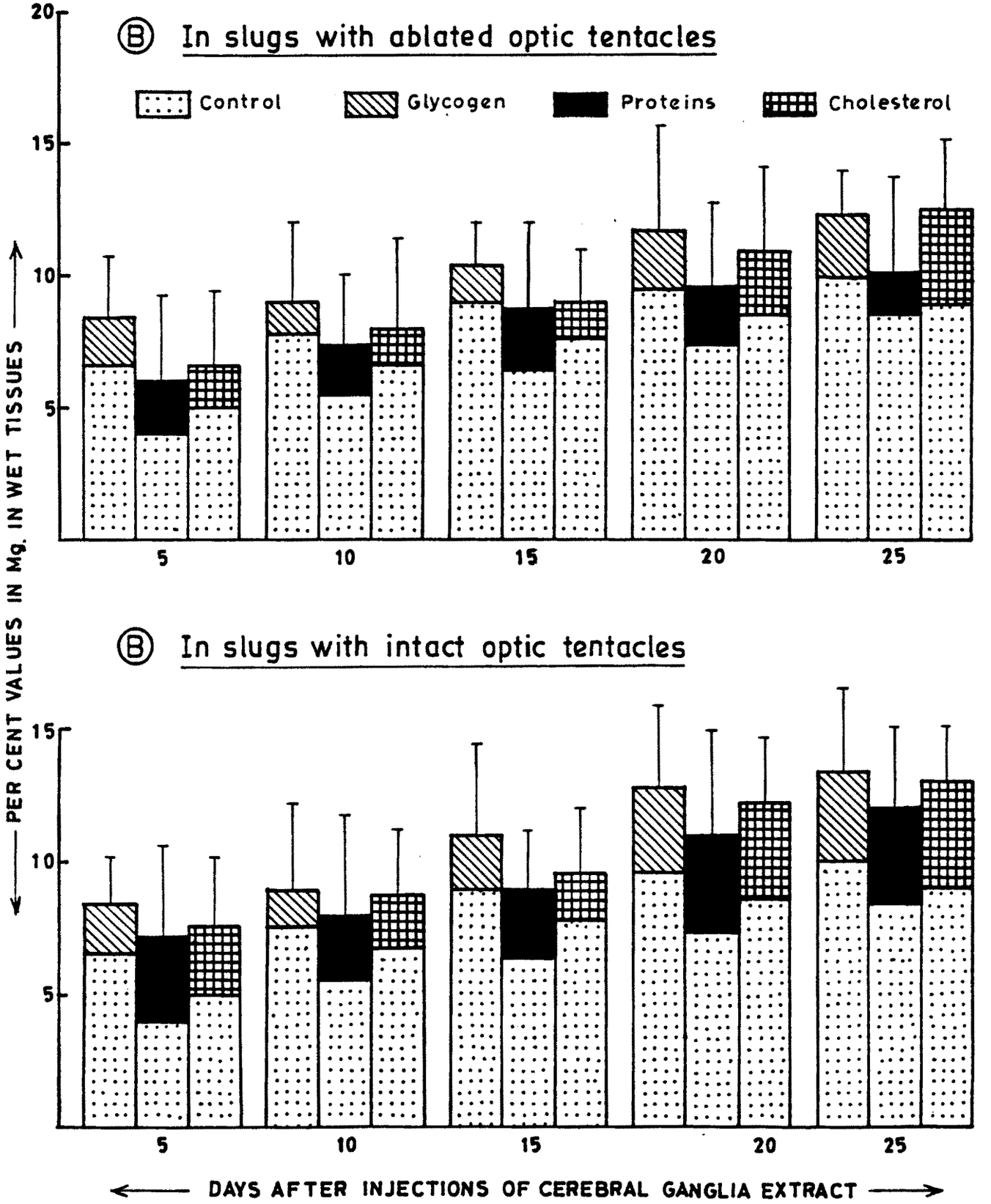
But after the injections of the extract of optic tentacles the values of these nutrients were increased. Per cent values of glycogen, proteins and cholesterol were 7.25 mg, 5.21 mg and 6 mg on the 5th day and through the intermediate values reached to 11 mg, 10.12 mg and 10.5 mg on the 25th day after injections in the group of slugs with ablated optic tentacles respectively. The per cent values of these nutrients were still increased in the group of slugs with the intact optic tentacles showing 7.96 mg, 6.02 mg and 6.52 mg on the 5th day and reaching to 12.50 mg, 11.24 mg and 11.59 mg on the 25th day after injections, respectively.

The results indicated the increase in the concentrations of glycogen, proteins and cholesterol in the penis due to the neurohormones of the optic tentacles.

C) **Effects of cerebral ganglionic neurohormones on the glycogen, proteins and cholesterol of penis** :

The neurohormones of cerebral ganglia increased per cent values of glycogen, proteins and cholesterol of the penis in both the group of slugs, with and without optic tentacles. These changes are recorded in Table No.9 and shown graphically in Graph No.11.

EFFECTS OF NEUROHORMONES IN CEREBRAL GANGLIA ON PENIS GLYCOGEN, PROTEINS & CHOLESTEROL OF *S. maculata*



GRAPH No.11

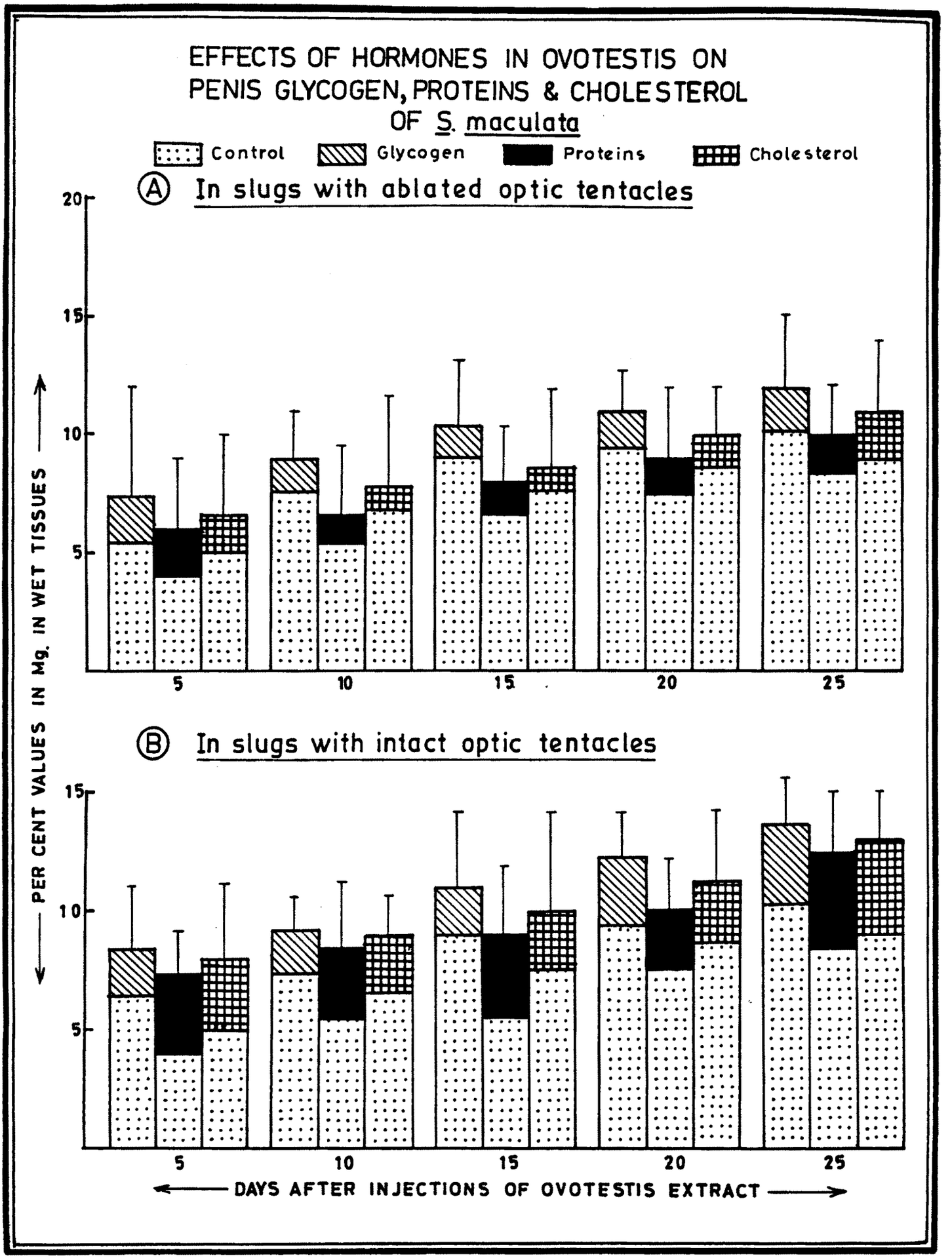
The increased glycogen, proteins and cholesterol values were reached upto 12.55 mg%, 10.25% and 12.66 mg% in the slugs with ablated optic tentacles and upto 13.75 mg%, 12 mg% and 13.03 mg% in the slugs with intact optic tentacles on the 25th day after the injections of extract of cerebral ganglia. The results indicated the increase in the concentration of glycogen, proteins and cholesterol in the penis due to the neurohormones of the cerebral ganglia. The increase being higher in the slugs with intact optic tentacles than the slugs with ablated optic tentacles.

D) Effects of ovotesticular hormones on the glycogen, proteins and cholesterol of the penis :

These effects are similar to the effects of neurohormones of the optic tentacles and cerebral ganglia. The results are recorded in Table No.9 and they are shown graphically in Graph No.12.

Per cent values of glycogen, proteins and cholesterol in the slugs with ablated optic tentacles were increased upto 12.09 mg, 10.62 mg and 11.50 mg, respectively. The values of these nutrients in the slugs with intact optic tentacles were increased still higher upto 13.92 mg, 12.54 mg and 13 mg, respectively, on the 25th day after the injections of extract of ovotestis.

Thus, the results indicated the increase in the concentration of glycogen, proteins and cholesterol in the penis of S. maculata under the effects of hormones elaborated in the ovotestis. These effects were higher in the slugs with intact optic tentacles than the slugs with ablated optic tentacles.


EFFECTS OF HORMONES IN OVOTESTIS ON
PENIS GLYCOGEN, PROTEINS & CHOLESTEROL
OF S. maculata
Control
Glycogen
Proteins
Cholesterol
(A) In slugs with ablated optic tentacles
(B) In slugs with intact optic tentacles
PER CENT VALUES IN Mg. IN WET TISSUES
20
15
10
5
5
10
15
20
25
DAYS AFTER INJECTIONS OF OVOTESTIS EXTRACT

GRAPH No. 12