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Neuroendocrine regulation of ovarian maturation in the Indian white prawn *Penaeus indicus* H. Milne Edwards

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ABSTRACT

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The control and regulation of ovarian maturation by neurosecretory elements in the eyestalk, brain and thoracic ganglia of *Penaeus indicus* have been examined. In the X-organ complex of the eyestalk, secretions from type A and B cells are apparently involved in inhibiting ovarian maturation, while in the brain and thoracic ganglia, secretions from type GN, A and B cells had a stimulatory effect on the ovary. The pyriform C cells were not involved with ovarian maturation. Both unilateral and bilateral eyestalk ablation resulted in precocious maturation of the ovary. The histology of the remaining eyestalk in unilaterally ablated animals revealed that all the NSCs in the X-organs were in a suppressed state and the sinus gland was devoid of granular aggregations. Abnormal behaviour was noticed in bilaterally ablated prawns and strangely few animals moulted with developing ovaries. Due to the synchronous occurrence of moulting and reproductive activities the probability of both gonad and moult inhibiting hormones being the same is discussed. In addition the relationship between gonadal maturation and the moult cycle has also been studied.

INTRODUCTION

In recent times the aquaculture of penaeids has received an added impetus by the application of the eyestalk ablation technique to obtain precocious maturation and spawning. It was in 1970 that maturation was first observed in an ablated *Penaeus duorarum* (Caillouet, 1972), almost 30 years after Panouse (1943) first obtained precocious maturation in *Leander serratus*. Since 1970, studies on induced maturation by eyestalk ablation in penaeids have multiplied (Alikunhi et al., 1975; Aquacop, 1975; Arnstein and Beard, 1975; Muthu and Laxminarayana, 1977; Lumare, 1979; Emmerson, 1980). Pres-

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ently around 14 penaeid species have been matured and spawned in captivity using this technique (Primavera, 1984).

In spite of the almost routine use of the technique of eyestalk ablation in penaeid prawn hatcheries, very little is known about the morphological and physiological changes that occur in the neuroendocrine system operating under these circumstances. In Decapoda, only few attempts have been made to correlate changes in the gonad with those in the neuroendocrine organs (Matsumoto, 1958; Perryman, 1969; Babu et al., 1980; Kulkarni and Nagabhushanam, 1980; Rao et al., 1981; Joshi, 1989). Such studies have added significance, especially in penaeids, as hatcheries are becoming increasingly dependent on endocrine manipulations to achieve their production targets.

The neurosecretory cell types and their cyclic secretory activity in *Penaeus indicus* have been reported earlier by Mohamed (1989). In the present investigation, attempts have been made to correlate the structural changes in the neurosecretory cells to different phases of ovarian maturation. Eyestalk ablation experiments were performed to study the NSCs concerned with the regulation and control of ovarian development. In addition, the relationship between gonadal maturity and the moult cycle has also been examined.

MATERIALS AND METHODS

Live adult females of *P. indicus* (140 mm and above) used in the study were collected from the sea off Cochin using short-duration otter trawls. The prawns were then segregated according to a five point maturity scale ranging from stage I (immature) to stage IV (mature) and stage V (spent) (King, 1948).

The various neuroendocrine centres, namely, optic, supraoesophageal, suboesophageal and thoracic ganglia, as well as the ovaries of the prawns in each maturity stage were dissected out and fixed in Bouin's fluid for 24–48 h and then processed for histological studies. Approximately 6–8 μm thick sections were cut and stained with Gomori's paraldehyde fuchsin (Kurup, 1972) for visualization of neurosecretory cells (NSCs) in the ganglia. Routine staining was, however, carried out with hematoxylin and eosin. Disulphide and sulphydryl groups of protein and glycogen are reported to be the principal components of the NSC in *P. indicus* (Mohamed, 1989). Hence histochemical tests with appropriate controls were carried out to detect the variation in these components in ganglia from all maturity stages according to Pearse (1968).

The NSCs in the optic, supraoesophageal, suboesophageal and thoracic ganglia of animals in different maturity stages were classified into three phases based on their secretory activity as described by Mohamed (1989). The phases were quiescent (Q), vacuolar (V) and secretory (S). The number of cells in each phase in all the ganglia was recorded for each maturity stage. Such cell

counts were made from three animals at same maturation stage and the average percentage was calculated.

Eyestalk ablation experiments

To study the role of eyestalk neurosecretory hormones on ovarian maturation, experiments were conducted at the Marine Prawn Hatchery Laboratory (MPHL) of CMFRI at Narakkal.

Female prawns above 140-mm TL obtained from the grow out ponds in the hatchery complex were used in the study after acclimatization to laboratory conditions for 48 h. Animals were maintained in 3-feet diameter collapsible plastic pools (Plasticrafts Corp., Bombay) with a capacity of 0.25 tons. Stored and settled seawater (salinity, 28 to 36 ppt; temperature, 27 to 30°C; pH, 8.0 to 8.2) was used and aeration was provided from an air-grid. Prawns were fed ad libitum with fresh or frozen clam meat. The uneaten food and faecal matter were siphoned out and 50% of the water was replaced daily.

Forty immature females of *P. indicus* in intermoult (C) and early premoult (D_0) stages were selected and divided into four experimental groups (I, II, III and IV) of ten animals each. In group I, prawns were maintained without any treatment for a period of 10 days and served as the intact control. In group II, animals were subjected to unilateral ablation of the right or left eyestalk. Both eyestalk of the prawns were removed in group III (bilateral eyestalk ablation). Eyestalk ablation was performed with the help of an electrocautery apparatus. Experimental animals were examined daily for signs of gonadal development. The development of the ovary could be clearly seen through the transparent dorsal cuticle and the formation of the triangular ovary in the first abdominal somite was taken as an indication of full maturity (stage IV). The number of days taken for attaining stage IV by each prawn was recorded as the latency period. Animals were sacrificed on reaching stage IV or after 10 days, whichever was earlier. The final moult stage was recorded and the gonads of each prawn were weighed for the determination of gonado-somatic index (GSI). Further the eyestalk, brain, suboesophageal and thoracic ganglia and the gonads were preserved in Bouin's fluid for histology.

In group IV unilateral eyestalk surgery was performed for all 10 prawns and simultaneously these prawns were administered an aqueous extract of fresh eyestalk. Eyestalk extract (from immature females) in the ratio of 2 eyestalks/0.2 ml was prepared by macerating fresh eyestalks in cold crustacean saline (3.4% NaCl, pH 7.4). The extracts were then centrifuged at 3000 rpm for 10 min and the supernatant was used for the injections. Each of the animals was injected once with 0.2 ml of the extract into the first abdominal somite using a hypodermic syringe. After an experimental period of 10 days, all the animals were sacrificed and their GSI and ova diameter were determined. Results of the experiments were compared using Student's *t*-test.

RESULTS

Relationship between NSC phases and maturity stages

The histological examination of the optic, supraoesophageal, suboesophageal and thoracic ganglia of wild females in different maturation stages revealed the significant changes taking place in the NSCs of these ganglia in relation to ovarian maturation. The mean percentage occurrence of NSCs in different phases of their secretory cycle (quiescent, vacuolar and secretory phases) in relation to ovarian development is given in Table 1.

In the eyestalk X-organ complex of immature females, more than 75% of the NSCs were in the physiologically active V and S phases (Fig. 1). In animals with fully mature gonads, the ratio of inactive to active cells was almost 1:1. In the spent stage, the cell percentage did not differ significantly from that of the mature stages. In marked contrast to the pattern observed in the eyestalk, in the supraoesophageal, suboesophageal and thoracic ganglia, the trend was the reverse. In the supraoesophageal ganglia of immature females a majority of the cells were in the Q phase and very few were in the S phase (Fig. 2). In the supraoesophageal of fully mature females, physiologically active NSCs together numbered more than 80% (Fig. 3). There was however, little change in this status in spent females. In the suboesophageal and thoracic ganglia, the changes in NSC phases were similar to that observed in the brain, although it was to a considerably lesser extent in the suboesophageal ganglion.

Histochemical responses of NSCs

Remarkable variations in the histochemical response of NSCs were observed in relation to the progress in ovarian maturity. The results of the histochemical tests on the optic, supraoesophageal and thoracic ganglia of immature, mature and spent females are presented in Table 2.

TABLE 1

Mean percentage occurrence of NSCs in different phases of the secretory cycle in relation to the female maturity stages in different neuroendocrine centres of wild *P. indicus*

Maturity stages	Eyestalk (X-organs)			Supraoesophageal			Suboesophageal			Thoracic		
	Q.Ph.	V.Ph.	S.Ph.	Q.Ph.	V.Ph.	S.Ph.	Q.Ph.	V.Ph.	S.Ph.	Q.Ph.	V.Ph.	S.Ph.
I–Immature	23.4	35.3	41.3	64.2	28.0	7.0	59.1	30.6	10.3	77.7	12.8	9.5
II–Early maturing	43.9	31.0	25.1	60.6	23.7	16.7	51.7	26.8	21.5	42.0	25.0	33.0
III–Late maturing	42.2	33.8	24.0	34.3	33.5	32.2	40.0	37.7	22.3	34.7	26.8	38.5
IV–Mature	47.3	29.6	23.1	19.8	32.2	48.0	25.5	31.6	42.9	13.9	30.7	55.4
V–Spent	51.5	24.7	23.8	19.0	33.9	47.1	23.9	32.0	44.1	14.2	30.2	55.6

Q.Ph. = quiescent phase; V.Ph. = vacuolar phase; S.Ph. = secretory phase.
Average of three observations.

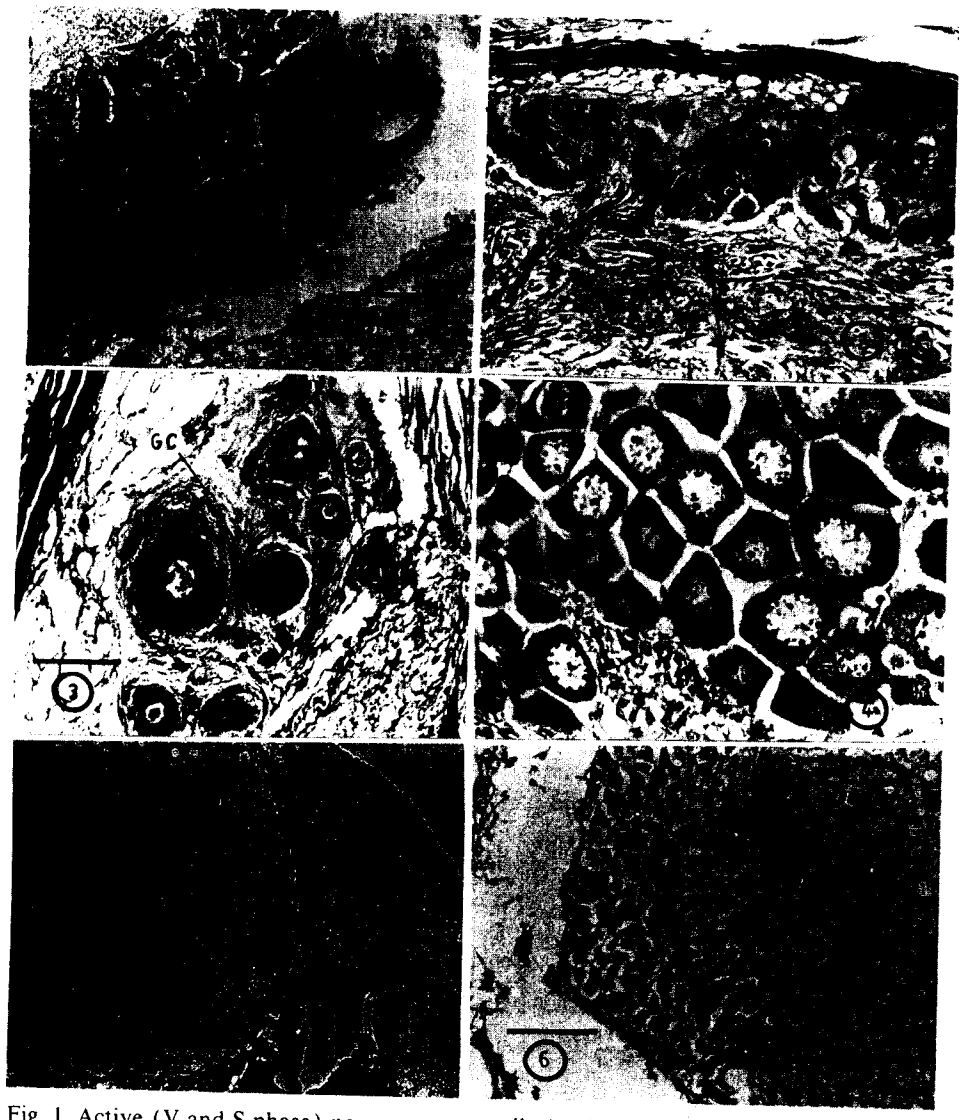

Fig. 1. Active (V and S phase) neurosecretory cells (A, B and C cells) in the MTGXO of an immature female. Bar equals 50 μm.
Fig. 2. Large number of quiescent (Q) phase NSCs in the supraoesophageal ganglion of an immature female. Bar equals 100 μm.
Fig. 3. Active NSCs in the supraoesophageal ganglion of a fully mature female. Note the A cell with hypertrophied glial cells (GC) and the intensely granular cytoplasm. Bar equals 50 μm.
Fig. 4. Previtellogenic oocytes in the ovary of an untreated (group I) immature female. Hematoxylin and eosin. Bar equals 50 μm.
Fig. 5. Ovary of a group II unilateral eyestalk ablated female having oocytes in fully mature state with peripheral cortical bodies (CB). N, nucleus. Hematoxylin and eosin. Bar equals 100 μm.
Fig. 6. MEGXO of the remaining eyestalk in a unilaterally ablated mature female. All cells have homogenous cytoplasm and are in the Q phase of the secretory cycle. ME, medulla externa. Bar equals 150 μm.

TABLE 2

Histochemical responses of the perikarya of different NSCs and the sinus gland in the optic, supraoesophageal and thoracic ganglia in relation to ovarian maturation in *P. indicus*

Ganglion	Histochemical tests	A cells			B cells			C cells			Sinus gland		
		IM	M	S	IM	M	S	IM	M	S	IM	M	S
Optic	1. Ferric–ferricyanide	++	+	++	+	±	±	+	±	+	+	±	±
	test—SH group												
	Control—Mercaptide	±	−	−	−	−	−	−	−	−	−	−	−
	2. PFAB test—S-S group	+	±	±	++	±	+	+	±	±	+++	+	++
	Control—Alcian blue alone	−	−	−	−	−	±	−	−	−	±	−	±
	3. PAS test—glycogen	+++	++	++	++	+	+	+	+	+	++	++	++
	Control—Diastase	+	−	−	+	−	−	±	−	−	±	±	±
		GN cells			A cells			B cells			C cells		
Supraoesophageal	1. Ferric–ferricyanide test	+	+	−	+	++	+	±	++	++	+	++	++
	Control—Mercaptide	±	±	−	±	±	±	±	−	±	±	−	±
	2. PFAB test	+	++	+	+	++	++	±	++	+	±	+	+
	Control—Alcian blue alone	−	−	±	±	−	±	−	−	−	−	−	−
	3. PAS test	+	++	+	++	++	+	+	+	±	+	+	±
	Control—Diastase	±	±	±	+	±	±	±	±	±	±	±	±
Thoracic	1. Ferric–ferricyanide test	±	++	+	+	+	±	+	++	+	±	++	+
	Control—Mercaptide	−	−	−	−	−	−	±	−	−	−	−	−
	2. PFAB test	++	+++	+	+	++	++	+	+++	±	+	++	±
	Control—Alcian blue alone	±	±	−	−	−		−	−	−	−	−	−
	3. PAS test	+	++	±	±	+	+	±	++	++	±	+	+
	Control—Diastase	−	±	−	−	−	−	−	±	±	−	±	±

Key: − = Negative reaction; ± = Very mild reaction of doubtful nature; + = Positive reaction; ++ = Strong positive reaction; +++ = Intense positive reaction. IM = immature (stage I); M = Mature (stage II, III and IV); S = Spent recovery (stage V).

In the optic ganglia, the NSCs displayed maximum positivity for sulphur-containing amino acids and glycogen in the immature stage and minimum positively in the mature and spent stages. A and B cells were the most active cell types in the X-organs by virtue of the magnitude of variation in the reactions. Maximum activity of the sinus gland was also observed in the immature stage. In the supraoesophageal and thoracic ganglia however, GN, A and B cells were the most active in relation to gonadal maturation.

Eyestalk ablation and ovarian maturation

Results of the experiments carried out to find the role of the X-organ sinus gland complex on ovarian maturation are summarized in Table 3.

In the experimental group I, untreated females, no ovarian development took place. During the 10 days of experiment, all the animals which were initially in intermoult (C) and/or early premoult (D_0) stages advanced to premoult and late premoult without moulting. Histologically the ovary showed the characteristics of an immature gonad with previtellogenic oocytes (Fig. 4). All group II animals exhibited significant increase ($P<0.01$) in their GSI and oocyte diameter as compared to group I animals. In this group, apart from an initial loss of equilibrium, no behavioural changes were observed after ablation although food intake was substantially increased. The latency period for maturation ranged from 4 to 9 days. Full maturity with oocytes in the vitellogenic phase was attained by 90% ot the experimental animals (Fig. 5). There was no significant change in the moult stage during the period.

The histology of the remaining eyestalk (the that was not ablated) revealed that all the NSCs in the MTGXO and MEGXO were in a suppressed state. The cytoplasm of these cells was uniformly agranular and homogenous, as is characteristic of Q phase NSCs (Fig. 6). The sinus gland of these animals lacked granular inclusions and stained lightly with aldehyde fuchsin (Fig. 7) when compared to the sinus gland of an immature female (Fig. 8). Further the majority of NSCs in the supraoesophageal and thoracic ganglia were in the V and S phases (Fig. 9).

TABLE 3

Details of eyestalk ablation experiments in relation to gonadal maturity in *P. indicus*

Expt. group	Mean total length (mm)	Mean latency period (days)	Moult stage		Mean GSI	Ova diameter range (μm)
			Initial	Final		
I	142.5	10	C/D_0	D_0D_{2-3}	0.488	20–100
II	146.7	5.9	C/D_0	C/D_0	5.699	164–347
III	142.3	3.7	C/D_0	$B/C/D_{2-3}$*	4.035	60–346
IV	145.4	10	C/D_0	$C/D_0/D_1$-	0.904	20–150

*Three prawns moulted once with developing ovaries and two died during the effort to moult.

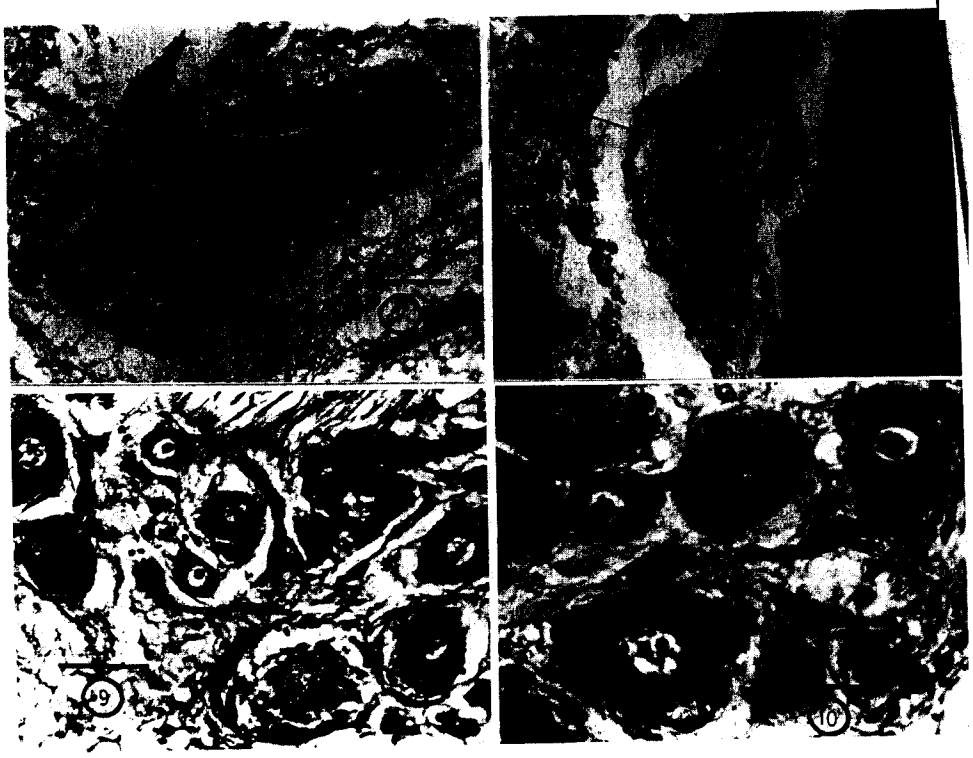

Fig. 7. The sinus gland of the same animal is lightly stained and devoid of granular inclusions IS, internal blood sinus; AX, axonal ending. Bar equals 100 μm.

Fig. 8. The sinus gland of an immature female with swollen axonal endings (AX) and secretory granules. IS, internal blood sinus. Bar equals 100 μm.

Fig. 9. Very active B cells (S phase) in the thoracic ganglion of a unilaterally ablated female in advanced stage of maturity. Bar equals 50 μm.

Fig. 10. Hyperactive B and C cells in the supraoesophageal ganglion of a bilaterally ablated mature female (group III). Bar equals 25 μm.

The bilateral eyestalk ablated animals (group III) showed significant behavioural abnormalities. Immediately after the extirpation of both the eyestalks, prawns were seen to swim at the surface in circles with abnormal speed Almost within a few hours, these prawns became dark red in colour due to the dispersion of chromatophores. Feeding was observed to be voracious. In this group 50% of the individuals attained full maturity. The average latency period was 3.7 days, which is considerable shorter than that observed in group II (5.9 days). Interestingly three prawns with developing ovaries moulted and two others with developing ovaries died during the moulting process. Moult

TABLE 4

Maturation in relation to moult cycle for wild females and unilateral and bilateral eye ablated females in *P. indicus*

Moult-stages		% occurrence of ripe (stage IV) females		
		Wild ($n=61$)	Unilateral eye ablation ($n=10$)	Bilateral eye ablation ($n=8$)
Postmoult	A	0	0	0
	B	0	0	12.5
Intermoult	C	18.03	10.0	25.0
Premoult	D_0	81.9	90.0	50.0
	D_1	0	0	0
	D_1	0	0	0
	D_1	0	0	0
	D_{2-3}	0	0	12.5

ing itself was abnormal as it was observed during daylight hours. The NSCs of cerebral and thoracic ganglia of these animals were in a hyperactive state (Fig. 10). Both V and S phase NSCs dominated in all the NSC groups in the ganglia.

In group IV where unilateral eyestalk ablated females were administered with an aqueous extract of the eyestalk, the mean GSI was estimated to be 0.904. This value was marginally higher than that of the control but was significantly lower ($P<0.01$) than that of groups II and III animals. After the 10-day latency period, the NSCs in all the ganglia showed characteristics similar to that existing in an immature female.

Relationship between gonadal maturation and moult cycle

The percentage occurrence of ripe (stage IV) females in different moult stages is given in Table 4. In samples obtained from the wild population more than 80% of the ripe females were in early premoult (D_0) stage. The remaining were in intermoult (C) stage. A similar pattern was also observed in unilateral eyestalk ablated females. In the bilateral eyestalk ablated group considerable variation in the above pattern was observed. Totally 75% of the animals were in D_0 and C stages. However, 12.5% of the mature females were in late premoult (D_{2-3}) which is the terminal stage prior to ecdysis. The remaining 12.5% were in late postmoult (B) stage. More importantly and perhaps uniquely, mature animals in B and C stages had moulted once with developing and developed ovaries.

DISCUSSION

In *P. indicus*, NSCs in V and S phases dominated in the MTGXO and MEGXO in the eyestalk of immature females. Subsequently in the ripe stage

Q phase NSCs dominated. The sinus gland was swollen with disulphide-rich secretory granules in the immature stage and it was devoid of granular inclusions in the mature stage. Similar cytological differences in the activity of the X-organ NSCs were correlated with the seasonal activity of the ovary in the shrimp, *Pandalus gracilis* (Aoto and Nishida, 1956), and in the freshwater crab, *Potamon dehaani* (Hanaoka and Otsu, 1957). Kulkarni and Nagabhushaman (1980) have shown in *Parapenaeopsis hardwickii* that the activity of the ovary (gonad) inhibiting hormone (GIH) was the highest in the eyestalks of females with inactive and spawned ovaries, whereas it was negligible in those at full vitellogenesis. However, in the present study, spent animals also had a large percentage of inactive NSCs. This is probably because of the small time-interval between stage IV and V and it could also explain the findings by Muthu(1983) that once *P. indicus* reaches reproductive maturity and spawns, it is able to undergo the process of rematuration within a short period of time.

The histological and histochemical results obtained in the present study showed that A and B cells in the X-organ complex of *P. indicus* were maximally active during the immature stage and therefore these cells may be responsible for the elaboration of a factor which inhibits the maturation of the gonad. Added evidence to this statement comes from the histological evaluation of the remaining eyestalk in prawns induced to mature by unilateral eyestalk ablation. All the NSCs in the MTGXO and MEGXO, particularly B cells, were in a suppressed state and the sinus gland was devoid of any secretory material.

Both unilateral and bilateral ablation of the eyestalk led to precocious maturation of the ovary and the same was inhibited by the injection of an eyestalk extract confirming that gonad inhibitory principles are present in the eyestalk. Also, in destalked male *P. japonicus*, an aqueous extract of the eyestalk of *P. vannamei* was found to inhibit the precocious development of the spermduct (Chim et al., 1983). Among female penaeids, eyestalk ablation has so far been synonymous with unilateral eyestalk ablation. Arnstein and Beard (1975) and Santiago (1977) observed that ablation of a single eyestalk was sufficient to induce maturation in *P. orientalis* and *P. monodon* respectively. Similarly in *P. indicus*, Muthu and Laxminarayana (1977) reported that unilateral eyestalk ablation was sufficient to induce precocious maturation as was observed during the present investigation. Bilateral ablation was observed to result in abnormal behaviour and incomplete maturation of the ovary. Identical results were obtained by Caillouet (1972), Alikunhi et al. (1975) and Muthu and Laxminarayana (1977).

In *P. indicus*, on occasions, bilateral ablation also led to moulting with maturing ovaries, an event which Adiyodi and Adiyodi (1970)reported as rare in crabs. Aquacop (1975) noted that they had never seen a penaeid moulting with developed ovaries except for a bilaterally ablated *P. aztecus*. Accelerated

moulting rate has been observed in *P. merguiensis* after bilateral ablation by Alikunhi et al., (1975), although they did not observe gonadal maturation side by side. The instance of synchronous occurrence of moulting and reproductive activities due to bilateral eyestalk ablation suggest the possibility that the hormones involved in inhibiting moulting and reproduction (MIH and GIH) are the same. Such an eventuality was also discussed by Adiyodi and Adiyodi (1970) when they argued the possibility of both GIH and MIH being chemically related molecules produced from the same NSCs in the X-organs. However, Quackenbush (1986) suggested that both MIH and GIH are separate peptides when he reviewed the progress made in their isolation. Eyestalk ablation is also known to shorten the moult cycle in crustaceans (Chang, 1985). However, Vijayan (1988) reported in immature females of *P. indicus* that though bilateral ablation shortened the moult cycle, unilateral ablation failed to do so. A comparison of the results of the uni- and bilateral ablation obtained in the present study seems to indicate that the emphasis during bilateral ablation (all inhibitory hormones removed) was toward moulting and during unilateral ablation (half of inhibitory hormones removed) it was toward maturation. Therefore, at least in penaeids, apart from the physiological state of the animal, the circulating inhibitory hormone level is also significant.

The NSCs of the supraoesophageal and thoracic ganglia and to a lesser extent the suboesophageal ganglion were maximally active in late maturing and mature *P. indicus*. In the crayfish *Procambarus simulans*, Perryman (1969) correlated the stage of ovarian development with varying amount of neurosecretory material in cell type III of the cerebral ganglion. Such a high secretory activity during ovarian maturation has also been observed in specific NSCs in the thoracic ganglion of *Macrobrachium lankesteri* (Rao et al., 1981) and *Potamon koolooense* (Joshi, 1989). The histochemical observation of the supraoesophageal and thoracic ganglia indicate that the secretions of the GN, A and B cells present in these ganglia may be responsible for the stimulatory effect on the ovary. Additional evidence for the elaboration of this stimulating factor comes from the observation of heightened neurosecretory activity in these ganglia after unilateral and bilateral ablation. Similarly in the crab *Menippe rumphii* bilateral eyestalk ablation resulted in the release of neurosecretory material from the NSCs of the brain and thoracic ganglion (Babu et al., 1980). Further they observed that the secretory activity of the thoracic ganglion more pronounced than the cerebral ganglion, suggesting the involvement of thoracic NSCs in reproduction. In *P. indicus* however, both cerebral and thoracic NSCs appear to be equally involved.

Crustaceans regulate gonadal and somatic growth to achieve an optimum balance between the two processes (Quackenbush, 1986), and based on the organisation of these two processes Charniaux-Cotton (1985) fitted shrimps into moulting type 2, wherein gonadal and somatic growth occur simultaneously. However, in prawns sampled from the wild population it was observed

that maturation almost always occurred in the early premoult stage. Not surprisingly, this is the moult stage in which the animal stays the longest (Vijayan, 1988). This indicates that penaeid prawns actually fit somewhere in between type 1 (where reproduction takes place during the long intermoult period) and type 2. Results obtained by Emmerson (1980) and Crocos and Kerr (1983) were also similar when they correlated events in the reproductive and moult cycle in *P. indicus* and *P. merguiensis* respectively. However, in the present study, this pattern was observed to be grossly disrupted in the bilaterally ablated group, although unilateral ablation seemingly did not affect it.

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