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Seasonal variations and the role of neurosecretory hormones on the androgenic gland of the prawn *Macrobrachium lamerrii*

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Abstract. The androgenic gland of *M. lamerrii* is situated near the seminal vesicle and along the vas deferens of the male prawns. Histologically it is made up of cords of cells which are arranged loosely. Histochemical analysis of the androgenic gland cells showed the presence of cystine/cysteins, protein bound amino acid groups. Androgenic gland of *M. lamerrii* shows signs of increased secretory activity during the sexually active phase of the male. In adult prawns, eyestalk ablation results in the hypertrophy of androgenic gland. Brain and thoracic ganglion extracts also showed enhanced secretory activity of androgenic gland and corresponding gonadal activity in the male prawns.

Keywords. Androgenic gland; eyestalk ablation; *Macrobrachium lamerrii*; eyestalk extract; brain extract; thoracic ganglion.

1. Introduction

The androgenic gland which secretes androgenic hormones has been established as the endocrine gland which is responsible for the differentiation of the primary, secondary sexual characters in the malacostracan crustaceans (Charniaux-Cotton 1960, 1964). Seasonal variations in the androgenic gland activity has been reported in the crustaceans (Hoffman 1968, 1969). The neuroendocrine control of androgenic gland by eyestalk ablation and eyestalk extract injections was experimented in crabs (Demeusy and Veillet 1958; Rangnekar *et al* 1971). The above literature shows that the freshwater prawns were neglected in this aspect which prompted us to note the seasonal cyclicity of androgenic gland and the role of neurosecretory hormones on the androgenic gland of the freshwater prawn, *Macrobrachium lamerrii*.

2. Material and methods

The androgenic glands (AG) used in the present study were obtained from intermoult (C) prawns. The AG were dissected out and kept in crustacean saline for morphological observations. For histological preparations, the AG were fixed in Bouins fluid and sectioned at 8 μm, stained in Gomori's chrome alum hematoxylin phloxine (CHP). The eyestalk ablation, eyestalk extract and brain, and thoracic ganglion extract injections were done as described by Diwan and Nagabhushanam (1974).

Histochemical nature of the androgenic gland was studied by treating the tissues in Susa, carnoy and alcoholic Bouins fixative for 24 hr. The tissues were dehydrated, paraffin embedded and sections (7–8 μ in thickness) were treated for histochemical tests

Table 1. Results of histochemical tests on the androgenic gland of *M. lamerrii.*

Tests	Cytoplasm	Nucleus
For proteins		
Mercuric bromophenol blue	+ +	+
Millons reaction (Bensley and Gersh modification)	–	–
Millons reaction (Baker modification)	–	–
Ninhydrin-Schiff method	+	+
Ferric cyanide method for -SH groups	–	–
Aldehyde fuchsin	+	±
For carbohydrates		
Best's carmine	–	–
Performic acid (Schiff method (PFAS))	–	–
Periodic acid-Schiff method (PAS)	±	±
For lipids		
Sudan black B	±	±

– = negative; ± = doubtful; + = positive; + + = intensity moderate.

as given in table 1. The histology of the testis was done by fixing the tissue in Bouin's fluid and staining the sections with Harri's haematoxylin eosin. The number of testicular follicles were counted.

3. Observations and results

3.1 *Histology and histochemistry of* AG

In the male *M. lamerrii* the AG was located near the terminal part of the seminal vesicle and extended over the vas deferens to which it is superficially attached (figure 1). The diameter of the AG measured at the seminal vesicle was 75 μm and near the vas deferens it is in the form of a diffused structure.

In whole mounts the AG looked transparent and the size varied with the reproductive stage of the animal. (21·8–90·5 μm). In histological preparations, the cords of the cells

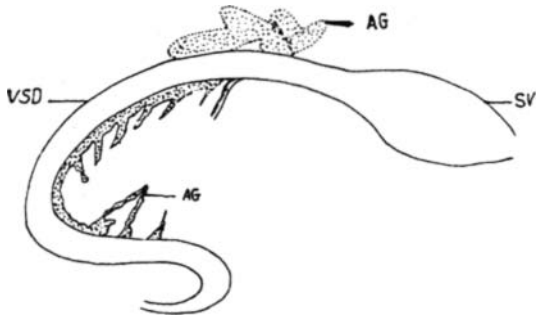

Figure 1. Gross morphology of androgenic gland. (AG-androgenic gland, SV-seminal vesicle, VSD-vas deferens.)

are loosely arranged, the cytoplasm in the cells scanty and the nuclei stained dark blue-black.

The histochemical tests revealed that the AG cells are positive to bromophenol blue and ferric ferricyanide and Millon's reaction failed to detect SH groups and tyrosine, but aldehyde fuchsin and ninhydrin tests gave positive results. The performic acid Schiff and Best's carmine tests gave negative reports.

In routine histological preparations, the gland cells appear almost identical and the cell boundaries can be hardly made out in light microscopy when the gland is active, the cells contain large amounts of CH positive granules. During inactive stages, signs of nuclear pycnosis becomes distinct.

3.2 *Seasonal variations in the AG*

The AG remained in a regressed state all through, May, June and July (table 1). Pycnotic and shrunken nuclei were observed throughout the gland. The gland cells very rarely showed the presence of fine basophilic granules. The AG started hypertrophy by August and September. The nuclei were large and the cells were multinucleolated and fine basophilic granules were observed scattered evenly in the cytoplasm. Nuclear pycnosis was rare in the cells. By October, November and December the hypertrophy of the AG increased steadily and during February the hypertrophied AG showed regional differences in appearance and histology also. Some areas in the gland showed basophilic granules in the cytoplasm and enlarged nuclei. The AG of March animals was largely comparable to those of February except that in some areas, the cells showed accumulations of granules into masses. By April the cytoplasmic granular bodies increased in size to form larger and larger units and nuclear pycnosis was increasingly evident. By May and June the AG as a whole atrophied significantly and the cytoplasmic granules became scarce and the cells started degenerating.

3.3 *Seasonal variations in the testicular follicles*

The testis showed cyclic changes and this was evident by the variation in the number of testicular follicles. The number of testicular follicles were highest during February. No testicular follicles appeared in May and June. From November onwards these showed a steep incline reaching maximum in February. By end of April the testis did not show any activity.

3.4 *Effect of neurosecretory hormones on AG*

Eyestalk ablation caused hypertrophy after 15 days of operation. This was evident by the increase in the cell diameters and also the nuclear diameters. In the eyestalk ablated individuals, the cell and nuclear diameters showed an enhancement from $0{\cdot}98 \pm 0{\cdot}29\,\mu$ to $2{\cdot}16 \pm 0{\cdot}10\,\mu$ and $0{\cdot}47 \pm 0{\cdot}02\,\mu$ to $1{\cdot}53 \pm 0{\cdot}07\,\mu$ respectively. When the eyestalk extract injection was given to the eyestalk ablated animals, there was a significant decrease in the cell and nuclear diameters (table 2). Central nervous tissue extracts when injected into normal and eyestalk ablated individuals brought about enhancement in

Table 2. Seasonal variation of androgenic gland activity.

Month	Size of AG ± SD (μ)	Secretory activity of AG	No. of testicular follicles ± SD
May	5·2 ± 1·2	No activity	No follicles
June	4·9 ± 0·9	No activity	No follicles
July	6·4 ± 0·7	No activity	3·2 ± 0·1
August	7·3 ± 0·2	Cells active	3·4 ± 0·2
September	10·4 ± 0·6	No. of cells increased	5·0 ± 0·4
October	*15·3 ± 0·8	Cells compactly arranged	7·6 ± 0·5*
November	*20·0 ± 0·5	Cytoplasm developed	12·8 ± 0·7*
December	*25·0 ± 0·9	Hypertrophy	19·8 ± 0·7*
January	*29·6 ± 0·1	Hypertrophy	21·2 ± 0·4*
February	*45·9 ± 0·6	Hypertrophy	39·2 ± 0·4*
March	*34·2 ± 0·7	Regression started	16·4 ± 0·2*
April	*30·1 ± 0·1	Regression started	14·2 ± 0·6*

* $P < 0{\cdot}05$.

the above parameters. However, this enhancement was more in the individuals who had eyestalk ablation than normal ones.

4. Discussion

The location and gross morphology of the AG of *M. lamerrii* is comparable to that of other decapod crustaceans (Charniaux-Cotton *et al* 1966). The AG showed its peak activity during February to March and it remained inactive from May to July. Gain in size of the gland, abundance of basophilic granules in the cytoplasm, increase in the size of nuclei and multinucleolated nature are some of the parameters taken into account to consider signs of increased secretory activity of the AG. All these cytological events in the gland cells occur when the male is in the sexually active phase. Seasonal changes in AG activity have been reported earlier in the crayfish, *Orconectes nais* (Carpenter and Deroos 1970). In the present study when the secretory activity of AG is correlated with the number of testicular follicles there appears to be a direct relationship (table 1).

Secretory activity of the AG in crustaceans is maintained by the influence of neurosecretory hormones (Charniaux-Cotton 1960, for review; Payen *et al* 1971). Eyestalk ablation brought about hypertrophy of AG in the marine crab, *Scylla serrata* (Rangnekar *et al* 1971). Inhibitory gonadotropins are produced in the neurosecretory complex of the eyestalks which inhibit testis development and AG. From the available literature it can be said that inhibitory action of gonadotropins is at first at AG level and further changes occur in the spermatogenic activity of crustaceans. Brain and thoracic ganglion extracts enhanced the spermatogenic activity and corresponding increase in androgenic gland activity. A stimulatory gonadotropin released from the photo-cerebrum was reported in *Orchestia gammarella* (Bounenfant 1967) and *Paratelphusa hydrodromous* (Adiyodi and Adiyodi 1974). Amato and Payen (1978) have suggested two types of specific neurohormones, one controlling the growth of the AG and the other

Table 3. Effect of eyestalk ablation and injection of central nervous tissue extracts on the androgenic gland activity.

Treatment	Cell diameter ± SD (μ)	Nuclear diameter ± SD (μ)
Normal (control)	5·98 ± 0·21	2·51 ± 0·50
Normal + eyestalk extract	5·71 ± 0·10	2·50 ± 0·10
Normal + brain extract	*12·07 ± 0·3	4·37 ± 0·30
Normal + thoracic ganglion extract	*12·87 ± 0·1	4·23 ± 0·06
Eyestalk ablated	*12·16 ± 0·1	4·53 ± 0·70
Eyestalk ablated + eyestalk extract	7·01 ± 0·5	2·52 ± 0·60
Eyestalk ablated + brain extract	*13·56 ± 0·4	1·57 ± 0·22
Eyestalk ablated + thoracic ganglion extract.	*13·69 ± 0·2	1·73 ± 0·10

*$P < 0·05$.

synthesis of male hormone. Further work on these lines is needed to establish the above facts in the freshwater prawn, *M. lamerrii.*

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