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METABOLIC EFFECTS OF EYESTALK ABLATION ON THE TOTAL PROTEIN AND LIPID OF THE PRAWN PENAEUS KERATHURUS (LEACH)

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ABSTRACT

Marked variation in moisture content could be detected with different length groups. Generally Prawns with immature stages have higher water percentage in their muscles than the mature ones. After ablation, the percentage of water content is greater than that of the unablated animal.

In the bilateral ablation of the eyestalk of the prawn Penaeus kerathurus (L), there was more lipid than protein in the ovary after 30 days postoperation.

The general decline in the total ovarian proteins of ablated animals might be attributed to its utilization on yolk formation. The inadequate availability of proteins following the ablation affects the intraovarian synthesis.

The remarkable postoperative augmentation of total ovarian lipids, which are encountered by an almost equivalent drop in hepatopancreatic lipids, may lead to a certain mode of transference of the hepatopancreatic lipids to the ovary, during the period of accelerated vitellogenesis following eysdtalk removal.

INTRODUCTION

There is a long history of eyestalk ablation research with crustaceans dating back to before the turn of the century. Until recently eyestalk ablation of crustaceans was always associated with high mortality (Abramowitz and Abramowitz, 1940 and Passano, 1960).

Water and ions in crustacean muscles are regulated with relation to a distinct process. In many species, the uptake and retention of water during proecdysis is an integral part of the moulting cycle.

The water balance hormone is clearly stored in and released from the sinus glands, but the neurosecretory center responsible for its manufacture has not yet been determined.

The control of water content during the moulting cycle is different in land crab Gecarcinus lateralis. This crab depends upon relatively infrequent rain showers and perhaps more frequent dew for its moisture (Bliss, 1968).

In normal Gecercinus *lateralis*, the foregut is permeable to water and ions in both directions during intermoult and after ecdysis. The diuretic hormone seems to control the permeability of the foregut, and hence presumbly the direction of the resulting net movement of ions and water (Boer et al., 1968).

Koch (1952) stated that, the ablated crab showed a greater increases in size after moulting, there was no increase in total protein in the tissues of those animals.

Neiland and Scheer (1953) reported that the removal of the sinus gland leads in starved crabs to a decrease in the protein content of the body. This decrease is pronounced in the female than in male.

Gonad inhibiting hormone inhibits the mobilisation to the ovary of yolk proteins in the crab Paratelphusa hydrodromous (Herbst) (Adiyodi, 1968). The same author comes to the conclusion that the decrease in the quantity of yolk protein in the plasma is a direct result of the eyestalk ablation.

Adiyodi and Adiyodi (1970) showed that eyestalks from males and females are essential in restoring normal maintenance of the gonad in both sexes.

Anilkumar and Adiyodi (1980) have studied the changes of some organic reserves due to eyestalk ablation of the crab Paratelphusa hydrodromous (Herbst) and reported that total protein in particular of precociously developed ovaries 15 and 30 days after eyestalk removal was far below that of normal ovaries.

According to Hilmy et al. (1986), the eyestalk amputation of the crab Portunus pelagicus (L.), resulted in decrease of the ovarian proteins and increase in lipids.

Kurup and Scheer (1966) and Gorell et al. (1972) have suggested that crustecdysone affects protein metabolism in the hepatopancreas, and this view is supported by the isolation from the hepatopancreas of two proteins which preferentially bind crustecdysone, and which are therefore likely to be specific receptor proteins for the hormone.

In many marine invertebrates, nutrients are stored in organs of the body other than the gonads, and are thought to be transferred to the gonads during gametogenesis (Giese, 1966).

Collatz (1969) said that lipid is transferred from hepatopancreas to the ovary in the crayfish. Herring (1973) stated that large quantities of the lipid, presumably from stores in the hepatopancreas, are transferred to the eggs in deep-living pelagic decapods.

The aim of this paper is to study the effect of eyestalk hormones deprivation on the chemical nature of the muscle, ovaries and hepatopancreas of the prawn **Penaeus kerathurus** (L).

MATERIAL AND METHODS

The present work is based on 50 immature and mature males and females of prawn in size from 100 to 180 mm brougth into the laboratory and maintained in a tank system supplied with re-circulated sea water through a biological filter. the system contained about 50 L of water, of which 20 L were changed weekly. The water temperature ranged from 20 to 23 ^{o}C during the experiment. The animals were fed daily on artemia.

After an acclimatization period, one eyestalk was removed, the second eyestalk being removed 24 hr later by holding the eyes with a forceps and cutting through the flexible membrane using a sterilized pair of scissors,. Penicillin was applied to prevent infection. After each operation, the prawn was put in filtered sea water for recovery. The ablated specimen was left in the air few seconds to prevent high mortality.

Fresh muscle samples were accurately weighed and then dried in an oven thermostatically regulated at 60^{o}C until they reached constant weights. The loss in water was taken as equivalent to the weight of water in the muscle sample. The water content is then expressed as a percentage of the wet weight.

Biochemical analyses were done after 5,10,15,20,30 days on the ablated and unablated animals. Total protein was determined by a new modification of the Lowry's method (Lowry et al., 1951) which was described by Tsuyoshi and Jaws (1978).

Total lipid was extracted and purified according to Bleigh and Dyer (1959). The technique involves the determination of total protein and total lipid of muscle, hepatopancreas and gonads and water content of muscle.

Data obtained were statistically treated according to Arkin and Colton (1963). T-test was usually used to evaluate the significance of the difference between the means.

RESULTS

A- Water Content :

Female immature stages have generally less water content than males of the same size group and females of the second size group (mature) were shown to have more water in their muscles than males. Marked variation in moisture content could be also detected with various stages of maturity. Generally, prawns belonging to the immature stages have higher percentage in their muscles than the larger animals. It is also observed that throughout the whole experimental period, the percentage of water content is greater in bilateral ablated animals than in the untreated ones (Table 1, & Fig. 1 A & B).

Table 1
RElationship between total body weight and total water content in both sexes of **Penaeus kerathurus** (Leach) in different stages of maturation.

Stage of maturation	Sex	Total body weight range (gm)	Mean moistur	
			Control (%)	Ablated (%)
1- Immature undeveloping	Male	5-13	83.4	87.8
	Female	5-10	80.6	85.3
2- Immature developing	Male	14-20	81.6	85.2
	Female	12-20	85.8	90.0
3- Mature nearly ripe	Male	22-30	71.4	78.3
	Female	22-30	78.8	81.2
4- Mature ripe	Male	35-60	76.1	80.0
	Female	35-60	71.6	75.8

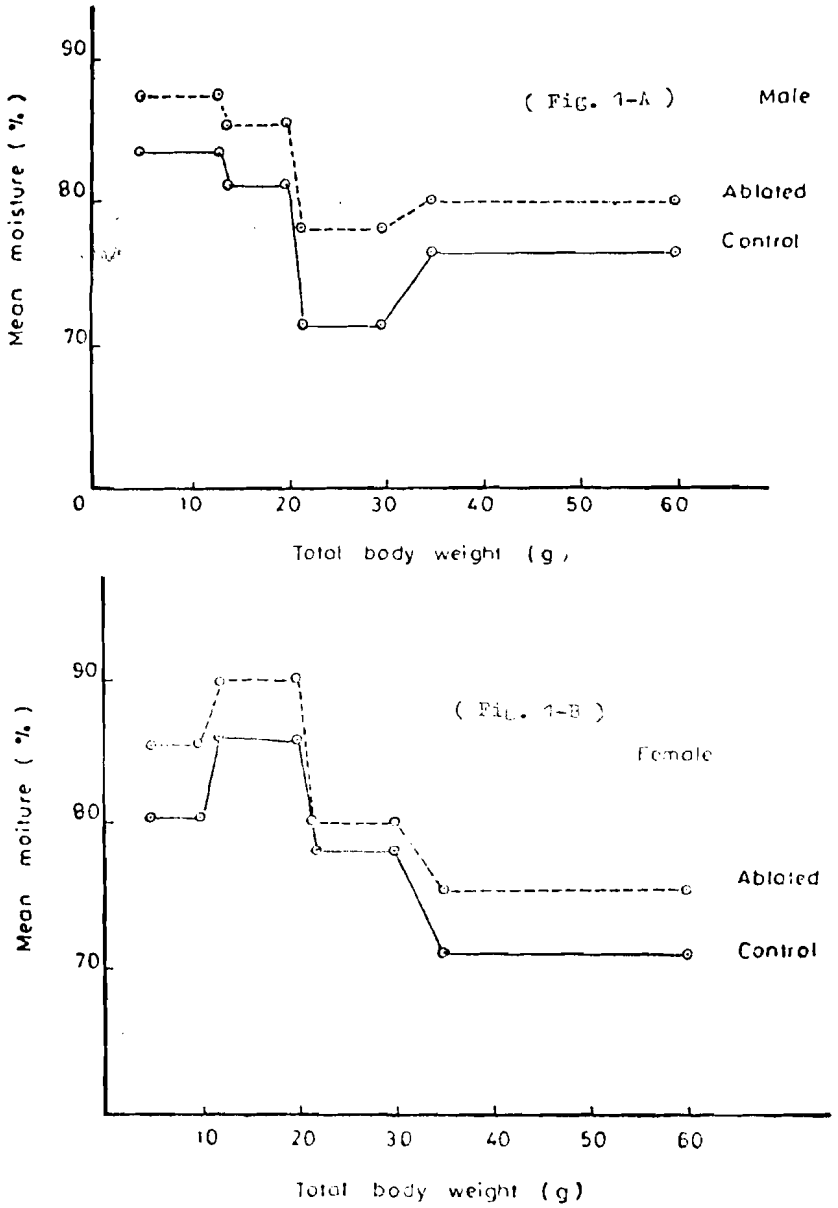

FIG. 1 (A & B)
Relationship between total body weight and content in both sexes of Penaeus kerathurus (Leach) in different stagesof maturity.

B- Total Protein :

Table 2 illustrates the results of total protein concentration in both ablated and control animals in the organs muscle, hepatopancreas and gonads. There is a striking difference in the total protein content of hepatopancreatic tissue between operated adult female prawns and the their matching controls. The levels of total protein content were drastically lower in operated animals than the levels obtained for controlled ones (Table 2). The previously mentioned results revealed that the lowest values were obtained 30 days after eyestalk extirpation.

Also the total protein of both muscles and ovaries showed fluctuations of lower and higher values with gradual decrease in the days postoperative. (Fig. 2).

C- Total Lipids :

Total lipid concentration in ablated adult female prawns was far below from that in normal intact animals. The variation of total lipid content of hepatopancreas of ablated animals registered a decrease down to 320.1 mg/g wet weight at the 5 days postoperation when compared with the values obtained for intact animals (280.0 mg/g wet weight). The lowest values were attained 30 days after ablation (210.5 mg/g wet weight), (Table 3 & (Fig. 3).

Table 2
Variations in total protein concentration of the muscle, hepatopancreas and ovary of adult prawn **Penaeus Kerathurus** (Leach under control and ablated conditions. All measurements were made on animals in each group

No.of days after operation	Muscle		Hepatopancreas		Ovary		levels of significanc *
	mean ±	S.D.	mean ±	S.D.	mean ±	S.D.	
Control	26.0	0.41	22.8	1.39	25.9	1.56	
5	22.9	1.27	20.9	0.37	20.3	0.45	H.S
10	24.2	1.19	18.7	0.37	15.6	0.84	H.S
15	21.3	1.23	16.9	0.22	18.3	0.41	H.S
20	19.8	0.38	16.3	0.36	14.2	0.45	H.S
30	18.3	0.73	15.1	0.29	13.8	0.42	H.S

Mean expresed as mg protein/100 mg tissue (dry weight).

± S.D. = Standard deviation.

* H.S. = High significance.

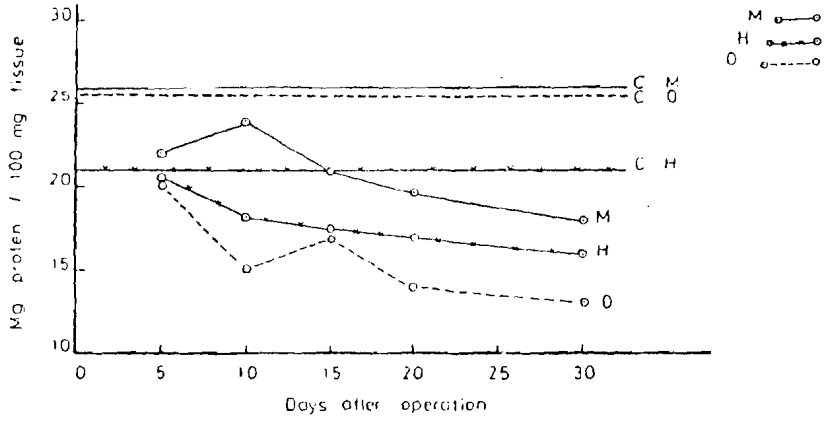

FIG. 2
Relationship between total protein concentration of the muscle (M), hepatopancreas (H) and ovary (O) of adult prawn Penaeusmuscle kerathurus (Leach) under control and ablated conditions. M, H, O = Ablated and CM, Ch, Co = Control

Marked variations postoperation could be detected in the levels of total lipid concentration in the muscle and ovary of the prawn Penaeus kerathurus (L). The lipid levels were higher during the whole experimental period in the ovaries than in control, while a decrease in muscle concentration was observed rapidly following the final operation (Table 3 & Fig. 3).

DISCUSSION

Various investigations revealed that the removal of eyestalks in crustacea induces great changes in the activity of many physiological and biochemical processes in the tissues of those animals.

The results of the analysis in the muscles of prawn Penaeus kerathurus (L) showed marked variations in the percentage of their moisture content.

The wide differences may be attributed to disturbance of the water metabolism due to differences in size, sex and maturity among individuals.

Passano (1953) obtained similar results, and claimed that they could be interpreted as differences in threshold. Water balance requires much less of the moulting inhibiting hormone to maintain normality than required to initiate proecdysis.

Table 3
Variations in total lipid concentration of muscle, hepatopancreas a ovary of adult female prawn **Penaeus kerathurus** (Leach) under contr and ablated conditions. All measurements were made on 5 animals i each group.

No.of days after operation	Muscle		Hepatopancreas		Ovary		levels of significanc
	mean	± S.D.	mean	± S.D.	mean	± S.D.	*
Control	110.0	0.85	280.0	4.18	180.0	0.93	
5	98.4	0.64	320.1	3.83	192.5	1.02	H.S
10	83.8	0.52	312.5	2.69	198.7	1.15	H.S
15	79.2	0.43	285.3	2.34	209.3	1.22	H.S
20	72.5	0.49	250.7	1.98	225.4	2.41	H.S
30	80.8	0.56	210.5	1.63	240.5	2.63	H.S

Mean expresed as mg lipid/mg tissue (dry weight).

± S.D. = Standard deviation.

* H.S. = High significance.

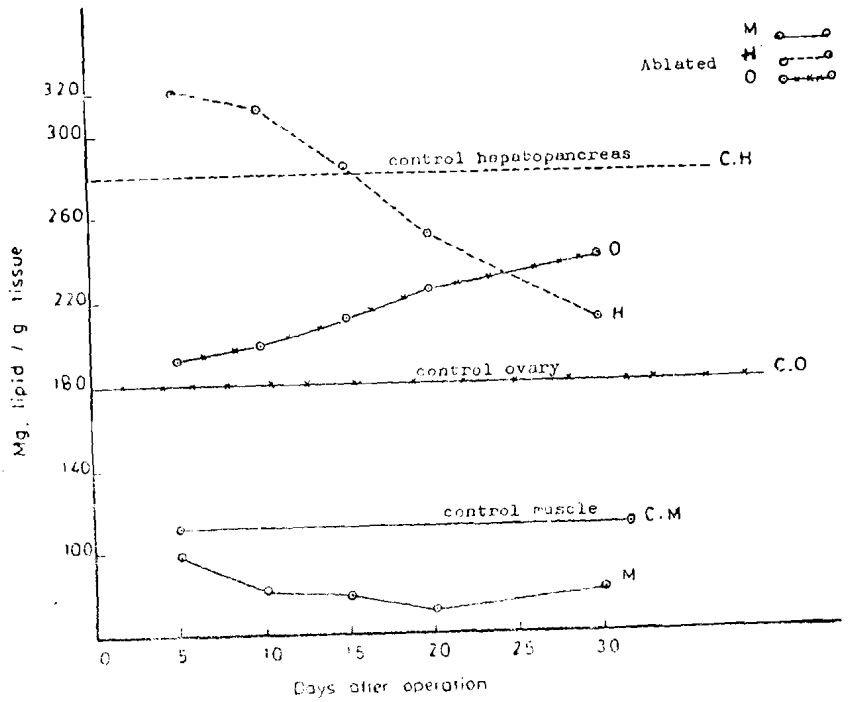

FIG. 3
Relationship between total lipid concentration of the muscle (M), hepatopancreas (H) and ovary (O) of adult female prawn Penaeus kerathurus (Leach) under control (C) and ablated condition.

Guyselman (1953) demonstrated a diurnal rhythm of water uptake in the crab Uca, and postulated as an explanation of some of his experimental data, that this is under the control of a hormone of the X-organ sinus gland complex. His data showed that the hormone responsible for water balance was not the same as the moult-inhibiting hormone.

However, the idea of the present work would concur with this view except that there is no reason to believe that the hormone is any other than the moult-inhibiting hormone itself. This probability tends to confirm the suggestion that the sinus gland serves only as a release center for a water balance regulating principle.

Koch (1952) showed that the greater intake of water at the moult in the animals lacking eyestalks is not a result of great formation of new tissues during the premoult, but simply due to a disturbed water metabolism. This has been confirmed in Carcinus means where it is possible to ablate the eyestalks 1-3 days before ecdysis and starve animals thereafter until they moult. Even under these conditions the water intake in the operated animals is far greater than in the controls (Francis et al., 1956). It is thus apparent that some factors in the eyestalks are concerned in the regulation of water metabolism.

It can be concluded that the water balance regulating hormone of the eyestalk is operating as an antidiuretic and indistinguished from the action of the antidiuretic principle of the posterior pituitary of vertebrates.

The total protein concentration was studied biochemically in homogenates of the muscle, hepatopancreas and ovaries from the prawn Penaeus kerathurus (L). The muscle protein concentration in the female prawn, shows an even greater and quite different pattern of change with ablated and control animals. Koch (1952) found that in fed animals there was no change after eyestalk ablation in the nitrogen and protein content of the tissues of the crab Eriocherir, except that after a moult the great amount of water taken in by the operated animals led to a corresponding apparent decrease in all the other constituents of the body.

Neiland and Scheer (1953) found that sinus gland removal led in starved crab **Hemigraspus nudus** to a decrease in the protein content of the body, more pronounced in the female than in male, suggesting that the sinus gland supplies a hormone necessary to restrain catabolism. Amputation of the tips of the eyes, however, depressed nitrogen output. Needham (1955) suggested that some factors present in the tips of the eyes normally antagonized the sinus gland complex.

Protein and lipid which are present in equal amounts by weight form the most prominent organic reserves in normal vitellogenesis ovaries of the crab Paratelphusa hydrodromous (Herbst) (Anilkumar & Adiyodi, 1980). In eyestalkless animals, the some found more lipid than protein in the ovary 30 days postoperation. The eyestalk ablation caused greater accumulation of lipid than protein in ovaries of destalked animals. The same authors have reported that in destalked adult **Paratelphusa**, there was a significant progressive increase in the level of total ovarian lipid, the highest values were obtained 45 days after eyestalk ablation.

A similar result was obtained in the present investigation for the prawn **Penaeus kerathurus** (L). Perhaps, the most important item in this study is that, following eyestalk amputation, the levels of total ovarian proteins gradually decreased while those of total ovarian lipids showed a marked progressive increase. This

discrepancy which was also observed in the contents of organic reserves of vitellogenesis of ovaries between the eyestalkless prawns and the controls could be due to either difficient yolk formation or an uneven distribution of yolk among the oocytes. It seems in general, that there is no complete correlation between decreased protein and increased lipid levels in operated animals.

Adiyodi and Adiyodi (1970) suggested that the oocyte development is controlled by the combination of the neurosecretion from the part intercerebralis of the brain and sinus gland hormone. The first affects the synthesis of protein in the hepatopancreas, and the second facilitates the yolk formation and once the growth is completed, their secretion is no longer used and they are accumulated.

It can be concluded that the changes of the above mentioned proteins are either an indirect result of hormone action or the endocrine secretions themselves.

Kulkarni Naga (1980) investigated the effect of bilateral eyestalk extirpation and injection of eyestalk, brain and thoracic ganglia extract on the ovarian development of eyestalkless and normal prawn Parapenaeopsis hardwickii. He comes to conclusion that there was a significant ($P > 0.05$) increase in total glycogen and fat concentration and decrease in protein content of treated prawn ovaries as compared to those of controls. Changes in the biochemical parameters of ovaries treated with unboiled eyestalk extract were not significantly ($P > 0.05$) different from those of controls. Our study revealed that it is possible that this general decline in total ovarian protein levels in destalked prawn might be due to either inadequate intraovarian synthesis or inadequate availability of proteins. Our results confirm the observation of Rao et al. (1981) and Hilmy et al. (1986). Rao et al (1981) have reported that during embryogensis, a marked change from protein of fat utilization occurs.

It has been observed in the present study, that there is a specific correlation between the total lipids of the ovaries of destalked prawns and those of the hepatopancreas. The increase in the levels of the formers is more or less equal to the decrease in the latters. This strongly suggests that there is a certain mode of transference of hepatopancreatic lipids into the ovary during the process of accelerated vitellogenesis following eyestalk removal.

This work also revealed that there is no evidence to exclude the possibility that the organic reserves accumulated in the storage depots of Penaeus kerathurus (L) may be utilized for reproduction. Moreover Lui et al. (1974) have given evidence that the ovaries of Decapod crustaceans do synthesize certain classes of lipids.

Gorell and Gilbert (1969 and 1971) stated that at least some aspects of hepatopancreas functions are controlled by hormones, but it is often uncertain whether crustecdysone or neurosecretory hormones are involved.

Mauviolt and Castell (1976) had reported for adult American lobsters, Homarus americanus, that after 95 or 174 days of accelerated weight gains, ablated lobsters had percent protein content equal to that of control lobsters. The only significant difference in composition was the slightly low level of lipid deposition in the hepatopancreas of ablated lobsters. They interpreted this as an indication of an accelerated rate of protein deposition and tissue synthesis in the ablated animals.

Our experiments on ablated prawn revealed a gradual decrease in the hepatopancreatic levels of lipid, most distinctly marked on the thirty postoperative days.

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