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SOME ASPECTS ON NITROGEN METABOLISM IN THE CRAB PORTUNUS PELAGICUS (LINNAEUS)

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ABSTRACT

The effects of extirpation of the eyestalks (ESX) from, and of injection of extracts of eyestalks (ESE) into eyestalkless crabs have been examined for the common nitrogenous components of the excreta and body tissues of the crab ***Portunus pelagicus*** *(L).*

Rate of elimination and concentration of protein, free amino acids, ribonucleic acid (RNA), deoxyribonucleic acid (DNA), non protein nitrogen (NPN), urea, uric and ammonia in various tissues have been determined during a period of 7 days after extirpation of eyestalk and of 12 hr. after injection of eyestalks extract on day 7th.

The concentration of protein N in the body has increased slightly after ESX. It is suggested from the data that an increased intake of about 20 mg N per animal per day would be required to maintain nitrogen balance after ESX, i.e to balance the increased excretory loss. Total protein N of the hemolymph increased significantly after ESX, suggesting that a factor in the eyestalks may restrain synthesis of hemolymph protein during the intermoult.

No amino acids nitrogen was detected in the excreta, but concentrations of free amino acids and of RNA in the various tissues have decreased after ESX but have returned to levels of unoperated controls by ESE. Therefore, it is concluded that an endocrine factor within the eyestalks that may normally restrains catabolism of amino

acids and of RNA during the intermoult stage of this species of crab, which minimizes nitrogen loss.

The nitrogen of urea, uric acid and ammonia all together and that of ammonia alone account for 62% & 56% of N eliminated respectively. The rate of elimination of total NPN and of each of the other products listed has increased to about five folds within 24 hr of ESX, but has remained above the level of control for 7 days afterwards. Injection of ESE is followed by a decrease below the level of saline injected ESX control within 12 hr but not to the level of control of each. Concentrations of the end-products in the different tissues have increased generally after ESX but have decreased after ESE.

INTRODUCTION

The processes of growth and maturation of organisms are controlled by the neuroendocrine system Yamaoka and Scheer, 1970). Neurohormones produced from the neurosecretory centres present in eyestalks are known to regulate a number of diverse physiological phenomena in Crustacea (Adiyodi and Adiyodi, 1970). The regulation of metabolite storage and utilization by neuroendocrine principles in the eyestalk have been reviewed (Honke and Scheer, 1970). There is a relatively large amount of work on the metabolism of carbohydrates in this groups, but very little information is available on nitrogen metabolism.

The processe of growth and development are regulated by the neuroendocrine system, centered in the eyestalks, and the y-organ or moult gland. (Yamaoka & Scheer 1970). Among the these processes is a substantial synthesis of protein during the intermoult period, between ecdyses (Renaud, 1949), and the incorporation of labelled amino acids into protein has been studied by Raghavaiah ***et al*** (1980) Evidence that the eyestalks of crustaceans produce factors influencing incorporation of amino acids into protein has been reported by Gorell & Gilbert (1971).

The investigation on ***Penaus kerathurus*** conducted by Abd El-Hamid (1989) revealed that the removal of the sinus gland leads to a decline in the total

ovarian proteins of the animals. The same auther concluded that the decrease in the quantity of yolk protein might be attributed to its utilization on yolk formation. The inadequate availability of proteins following the ablation affects the intraovarian synthesis.

Hashem ***et al.***, (1991) showed that unilateral eyestalk ablation is sufficient to induce maturation and spawning in ***Penaus kerathrus***, where as the bilateral ablation results in high mortalities. On the other hand, Abd El-Hamid (1994) pointd out that the extirpation of eyestalks caused a loss in that ribonucleic acid content of the hepatopancreas and thus a substance in the eyestalk might control the synthesis of RNA.

The present investigation was conducted to study the effect of extirpation of the eyestalks (ESSX) and of injection of eyestalks extract (ESE) on the concentration of major nitrogenous components within various tissues of the crab ***Portnuus pelagicus***. The ESX is classical operation of crustacean endocrinology with removal of X-organ, a neurohaemal organ containing the neurohaemal organ containing the neuron endinges of the neuroendocrine system. It releases the moult gland (Y-organ) from inhibition and thus initiates processes leading to moulting, and is known to influence various aspects of metabolism of nitrogen and of carbohydrates (McWhinnie and Mohrherr, 1970; Chu and Chow, 1992). The endocrine nature of these effects has not, in general, been demoonstrated by comparison of the effects of extirpation of the eyestalks with those of injection of extracts of eyestalks.

The important of this investigation will lead in the future to continue this experiment and to broaden its scope to enventually ensure an adequa and continuing supply of spawners for hatchey operations and consequently of fry for the expending industry. Improved ablation techniques, suitable age at which the operation take place, the best solvent for extraction of the hormonal eyestalk and nutritional requirements during maturation reduced mortality. Future success in these investigations and reliable mass propagation of fry at all times of the year becoming closer to reality will provide the necessary impetus for increasing production.

MATERIAL AND METHODS

Sixty adult specimens of both sexes of ***Portunus pelagicus*** (L) of mean weight 150.0-200.0g were collected alive from Abu-Kir bay (east of Alexandria) during the period from October to April of year (1993-1994). The crabs were maintained in the laboratory condition of national institute of Oceanography and Fisheries for a week before the commencement of the experiment, to acclimatize them to the laboratory conditions. They were kept in glass tank (70 x 100 x 50 Cm) with sufficient quantity of aerated and filtered sea water (with 32% salinity and average temperture of 25°C).

Extirpation of eyestalks was performed by cutting off the organ at its base with a sterilized pair of scissors; penicillin was applied to prevent bacterial infection. Evestalkless animals were removed from the experimental group for dissection and analysis at intervals of 24, 48, 72 hr & 5 or 7 days after operation. An extract of the eyestalks in ethanol was praoared according to the method of Silverthorn (1976). This extract was injected in a dose equivalent to two eyestalks (50 µl), through the articulating membrance at the base of the coxa of the fourth pair of walking legs. The injection was made on 7th day after ESX and animals were kept 12 hr before they removed for dissection and analysis. Unoperated controls for the eyestalkless series were removed from the experimental group at the same time as others when removed for ESX. Saline controls (SAL) for injection of ESE were prepared by injection of Harreveld's saline (van Harreveld, 1936) at the same time when others were injected with ESE. For analysis, body weight was determined to the nearest gram ad a sample of hemolymph was aspairated by plastic syringe through the arthrodial membrance of a limb joint. Muscles hepatopancreas, hypodermis and gills were then quickly and carefully removed out, weighed separately and analyzed individually according to the procedures outlined below.

For collection of materials eliminated into ambient medium, animals were transferred into a measured volume of water contained penicillin to minimize bacterial activity for 6-7 hr. The results of analysis of the medium were then used to calculate rates of elimination as mg of product per animal per 24 hr. Those animals injected wity ESE or SAL were injected twice at intervals of 12 hr before the beginning of excreta collection.

Analytical Methods

Total protein was determined by a modified of the lowey's method as described by Tsuyoshi and Jaws (1978). Ribonucleic acid (RNA) was estimated according to the method of Glick (1966), whereas deoxyribonuncleic acid (DNA) was measured by diphenylamine method of Giles and Myers (1965).

The content of substances reacting with ninhydrin (TNPS) was determined by the methods of Moore and Stein (1968). The photometric readings were compared with a standard curve for pure tyrosine, and converted to mg of amino acid on the assumption of a mixture of the composition given by Schoffeniels and Gilles (1970) for hemolymph or muscle of the crayfish ***Astacus fluviatilis***. Total non protein nitrogen (NPN) was measured in each ambient and various tissues by a modification of the kjeldahl method (Oser, 1965).

Urea nitrogen and uric acid were determined in a sample of ambient medium following the methods of Foster and Hocholzer (1971), and Oser (1965) respectively.

Ammonia was estimated in the ambient medium by direct treatment with Nessler's reagent before photometric measurements (Oser, 1965).

Results of analyses are expresed as mg/g wet weight of solid tissue or mg/100 ml of hemolymph. Statistical comparisons have been made by the method of confidence limits, as used by Scheer and Langford (1976) and Scheer (1979).

RESULTS

Total Proteins

Means of total protein concentrations varied among different tissues. But within the relativey short period of these experiments, there was a little consistent effect of eyestalk extirpation or eyestalk injection on protein content analysed (Table 1). Protein concentration in the hemolymph has increased somewhat after eyestalk removal, and has reached values statistically different from unoperated control's level only on postoperative 5th and 7th day. Whereas, proteins in gills and hepatopancreas were significantly lower than

Table (1): Total protein concentration in the different tissues of ***Portunus pelagicus*** (Linnaeus) as onfluenced by extripation of and injection with, extract of eyestalk. (Means and 1% confidence limits for groups of ten animals).

	days after extirpation						
	Control					**SAL**	**SES**
Tissues	**0**	**1**	**2**	**3**	**5**	**7**	**7**
Muscle	226.0	226.2*	213.0*	212.2*	215*	220.1*	225.0*
mg/g	± 9.5	± 10.2	± 5.1	± 8.0	± 7.3	± 8.3	± 15.0
hepato	130.1	121.0*	116.2*	103.9	118.2*	110.8*	122.0*
mg/g	± 4.3	± 7.3	8.3	± 10.6	± 11.3	± 3.4	± 5.5
hemolymph	37.32	44.6*	48.83+*	49.65+	72.0+	78.21+	55.1
mg/100 ml	± 10.4	± 9.8	± 7.7+	± 4.3	19.61	20.12	4.2
hypodermis	170.2	134.2*	125.2*	128.3	131.6*	138.6*	164.5ss
mg/g	± 4.2	± 14.2	± 2.2	± 8.6	± 8.6	± 8.2	5.2
gills	164.2	160.7*	142.0*	130.1	140.4**	127.2++	143.2ss
mg/g	± 21.3	± 2.7	± 8.1	±10.1	± 20.1	± 6.1	± 5.1

* Mean value within confidence interval for control.
± > control + < control SS > saline injected control (SAL)
± S.D. (standard deviation)

controls level, on day 7th. After the injection with hormone extract from eyestalk, concentration of muscles protein has not statistically changes from intact though is was higher than SAL in both gills and hypodermis.

Amino acids

The values of amino acids concentration in all tissues are shown in Table (2). Maximum concentrations were noted in hemolymph and muscles. ESX was followed by an immediate and continuous increase in amino acids content in hemolymph. In the contrary in all other solid tissues except gills, an immediate and continuous decrease was traced (Table 2). In gills, on the other hand, a decrease in amino acids concentration was noted on day 1^{st} & 2^{nd} after ESX. Injection of ESE was followed by an increase above SAL of control in all the tissues examined except hemolymph and gills. No amino acids could be detected in the ambient medium

Ribonucleic acid (RNA)

After eyestalk extirpation an immediate decrease in RNA concentration in hepatopancreas and gills, and a smaller but significant decrease in hypodermis was observed (Table 3). In these three tissues, ESE resulted in an increase above SAL's level but has not reached that of unoperated control. Therein, the concentration of RNA is used as an index of protein synthesis. The results for gills are similar to those for protein concentrations in hepatopancreas. The muslce and hemolymph are inconsistent for hepatopancreas, where the decrease in RNA was associated with change in protein.

Deoxyribouncleic acid (DNA)

The only effect noted of eyestalk extirpation (ESX) on DNA concentrations was a delayed, but significant increase of nearly four-folds in hemolymph on day 7th after ESX (Table 4). The concentration of DNA in the hemolymph like that of RNA is only about 1% of that in the cellular tissues. As DNA represents nuclear material, RNA and DNA must represent the cellular components of hemolymph (hemocytes). Injection with eyestalk hormone has decreased hemolymph DNA below SAL's level, but did not return it immediately to control's level. The inference that these changes are due to changes in the number of hemocytes requires independent verification, since they were not parallel with the changes in RNA.

Table (2): Total ninhydrin-positive substance (as mg of amino acid, and as amino acid N% of total NPN.

Tissue	*days after extirpation*						
	Control 0	**1**	**2**	**3**	**5**	**SAL 7**	**ESE 7**
Muscles	31.59	21.11++	20.11++	19.2++	22.12++	21.6++	27.9ss
(mg/g)	± 1.68	± 2.91	± 1.95	± 2.3	± 2.5	± 1.9	± 2.6
N (% NPN)	100	98	87	79	85	75	78
hepatopancreas	14.64	7.53++	8.92++	7.95	9.97++	6.96++	13.75
(mg/g)	± 3.2	± 0.92	± 1.2	± 1.22	± 1.12	± 1.1	± 1.5
N (% NPN)	39.7	18.2	19.6	17.2	21.6	17.8	26.4
hemolymph	11.54	31.62+	38.94+	35.6+	39.2+	35.92+	34.2+
(mg/100 ml)	± 3.21	± 4.01	± 3.91	± 4.01	± 3.1	32.8	± 2.1
N (% NPN)	39	35.8	33.6	38.8	43.4	32.7	49.1
hypodermis	13.23	6.24++	5.97++	6.84++	7.44++	3.87++	13.23ss
(mg/g)	± 0.68	± 0.42	± 1.1	± 1.1	± 0.72	± 5.1	± 1.0
N (% NPN)	27.1	11.2	10	12.4	13.7	6.7	37.7
gills	4.58	2.04++	2.09++	3.81	4.11+	4.06+	4.46
(mg/g)	± 0.06	± 0.50	± 0.45	± 0.51	± 0.40	± 0.17	± 0.53
N (% NPN)	19.6	7.7	5.8	10	9.9	9.2	9.0

* Mean value within confidence interval of control

\+ > controls ++ < saline - injected control (SAL)

Table (3): Values of ribonucleic acid (RNA) concentration under the effect of extirpation or injection with hormonal extract the crab ***Portunus pelagicus (L).***

	days after extirpation						
	Control					**SAL**	**SES**
Tissues	**0**	**1**	**2**	**3**	**5**	**7**	**7**
Muscle	5.52	6.19	5.11*	4.69	5.5*	5.27*	5.22*
mg/g	± 0.61	± 0.75	± 0.69	± 0.50	± 0.41	± 0.36	± 0.12
hepato	11.37	6.83+	6.07+	5.31+	5.21+	3.61+	9.39++
mg/g	± 2.32	± 1.92	± 0.32	± 1.78	± 1.78	± 2.01	± 0.55
hemolymph	9.22	10.96	11.66	10.27*	11.04	8.66*	9.50
mg/100 ml	± 1.5	1.22	1.35	1.46	2.40	1.14	0.50
hypodermis	6.59	4.71+	5.78	5.63	5.48+	4.25+	5.69
mg/g	± 0.53	± 0.45	± 0.60	± 0.50	± 0.30	±0.70	0.41++
gills	7.83	6.37+	6.25+	4.72+	4.84+	4.30+	6.51++
mg/g	± 0.91	± 0.33	± 0.47	±0.52	± 0.30	± 0.41	± 0.4

* Mean value within confidence interval for unoperated aimals (control).
+< controls ++> saline - injected control (SAL)
Unop = Unoperated control

Table 4): Relationship between deoxyribonucleic acid conceutration (DNA) in different tissues and injection with hormonal extract or extirpation of eyestalk on the crab ***Portunus pelagicus (L).***

	days after extirpation						
	Control					**SAL**	**SES**
Tissues	**0**	**1**	**2**	**3**	**5**	**7**	**7**
Muscle	1.97	2.36	2.55	2.09*	2.16*	0.83*	0.99*
mg/g	± 0.21	± 0.33	± 0.42	± 0.21	± 0.31	± 0.12	± 0.11
hepato	3.36	3.23*	3.86*	3.16*	3.12*	3.18*	3.29*
mg/g	± 0.68	± 0.44	± 0.66	± 0.34	± 0.56	± 0.52	± 0.55
hemolymph	4.33	5.08	5.19	8.04+	10.16+	13.4	9.39++
mg/100 ml	± 0.79	± 1.55	± 0.87	± 1.73	± 1.01	± 1.98	± 1.52
hypodermis	1.99	1.79*	1.61*	1.83*	1.69*	1.55	1.85
mg/g	± 0.41	± 0.26	± 0.30	± 0.35	± 0.31	± 0.21	+ 0.2
gills	1.34	1.49*	1.30*	1.67*	1.13*	1.1*	1.38*
mg/g	± 0.5	± 0.21	± 0.31	± 0.3	± 0.2	± 0.3	± 0.30

* Mean value within confidence interval for unoperated aimals (control).
+> controls ++> saline - injected control (SAL)
Unop = Unoperated control

Solubility of eyestalk factors

A comparison of two eyestalk extracts, prepared by either water or ethanol is summarized in (Table 5). The effect of these two types of extraction on concentration of amino acids and of total proteins in the different tissues, were compared as well. No significant effect was evidenced for boiled extract or for acetone extracts, and these results are not included in the (Table 5) Evidently, both water and alcohol extracts are active factor in increasing protein concentration of hepatopancreas and gills. The alcoholic extract used in (Table 2) acted on hepatopancreas and muscles. These results suggest the occurrence of several different factors in the eyestalk, with differential effect on the processes and tissues studied, hence more precise means of separation are required for further studies.

Non protein nitrogen (NPN)

From the results given in (Table 6) there was no significant difference among experimental treatments in NPN of hemolymph following ESE. However, NPN of hypodermis decreased below the level of both control groups. On the other hand, ESE has resulted in an increase in nonprotein nitrogen for hepatopancreas, muscles and gills to about unoperated control's level but not above SAL's level. Therefore, it is evident that the rate of elimination of NPN has increased nearly to five-folds within 24 hr after ESX, and remained high for 7 days afterwards. Injection of ESXE on day 7th was followed, within 12hr, by a decrease to more than 50% below the levels of saline-injected controls (SAL), but not to the level of controls. The increase in the rate of elimination after ESX was not reflected significantly as variations in the concentration of total nonprotein nitrogen in the different tissues.

Urea

Table (7) shows that, urea nitrogen is a relative minor component of total non-protein nitrogen, making up 5-10% of elimiated nitrogen and an even smaller proportion of the nitrogen within other tissues. Concentration of urea has increased after ESX in all tissues, but in an immediate and continuous way for only in hypodermis and gills. Following ESE, a decrease below SAL's to unoperated control level were evident in all tissues examined except hepatopancreas and hypodermis. The trend of variation after ESX is nevertheless parallel to those of NPN and of ammonia. The mean rate of urea elimination has increased nearly to eight-folds within 24 hr after ESX and

Table (5): Effect of aqueous and ethanolic extracts of eyestalks of ***Portunus pelagicus*** on concentration of total ninhydrine positive substances (TNPS, tyrosine equivalents) and of protein tissues.

days after extirpation						
Tissue	**Saline injected**		**Aquaous extract**		**Ethanolic extracts**	
	TNPS	**Protein**	**TNPS**	**Protein**	**TNPS**	**Protein**
Muscle	15.85	201.0	18.36	215.0	2.1.61	221.0
mg/g	± 2.83	± 13.9	± 2.81	± 12.2	± 2.1	± 10.1
hepatopancreas	11.21	83.90	13.51	111.0*	18.5	123.0
mg/g	± 3.21	± 3.2	± 3.21	± 8.2	± 4.0	± 9.2
hemolymph	54.92	70.27	50.32	58.61	48.82	50.1
mg/100 ml	± 7.21	± 8.7	± 7.2	± 8.2	± 5.6	± 6.0
gill	5.58	120.6	5.2	150.2*	7.9	150.1*
mg/g	± 1.12	± 8.9	± 1.3	± 12.1	± 2.1	± 6.1
gills	7.09	130.41	15.11	150.1	20.1*	163.1*
mg/g	± 1.81	± 8.1	± 3.1	± 10.1	± 4.5	± 10.2

* Mean ± 1% confidence limits for groups 5).
* > controls
± Standard deviation

Table (6): Effect of extirpation and injection of eyestalks extract on total non protein nitrogen and rate of elimination in different tissues of ***Portunus pelagicus (L).***

	days after extirpation						
	Control					**SAL**	**SES**
Tissues	**0**	**1**	**2**	**3**	**5**	**7**	**7**
Muscle (mg/g)	5.49	5.43*	5.76*	6.12*	6.42*	7.1	8.76+
± S.D.	± 1.07	± 1.5	± 1.42	± 1.48	1.62	±1.67	± 0.84
hepatopancreas	16.59	16.91*	16.94*	17.65	17.60	17.47*	18.95+
(mg/g)	± 0.57	1.72	1.14	1.25	1.28	1.28	± 0.61
hemolymph	11.55	12.45*	13.85	13.16	12.75*	15.35	10.15
(mg/100 ml)	± 1.41	± 4.53	± 4.46	± 4.41	± 3.72	± 4.1	± 1.8
hypodermis	7.58	8.79*	9.35	8.66*	8.58*	9.44	5.54
(mg/g)	± 1.2	± 1.51	± 1.8	± 1.8	± 2.1	± 1.71	0.42++
gills	3.06	4.68*	5.78	5.05	6.39	6.46	7.16+
(mg/g)	± 1.4	± 1.42	± 1.6	±1.91	± 1.82	± 1.93	± 0.91
Rate of elimination	6.1	26.93*	26.29	24.22	20.89	26.21	11.87+
(mg/animal/day)	1.4	1.42	1.6	1.91	1.182	1.93	0.91

* Mean value within confidence interval for control.

+ > controls

++ < saline - injected control (SAL)

Table (7): variations in urea concentration in different tissues of adult crab ***Protunus pelagicus*** (L) under the ablated and hormonal extract injected conditions.

	days after extirpation						
	Control					**SAL**	**SES**
Tissues	**0**	**1**	**2**	**3**	**5**	**7**	**7**
Muscle	2.30	2.70*	2.56*	2.90	5.1+	9.90+	3.62
(mg/g)	± 0.03	± 0.08	± 0.03	± 0.03	± 0.1	± 0.18	0.02++
N (% NPN)	1.7	2.6	2.6	3.4	3.0	3.7	7.3
hepatopancreas	4.8	6.4	5.9+	6.7	13.9	11.9+	10.2+
(mg/g)	± 0.10	0.08	± 0.20	± 0.10	± 0.19	± 0.30	± 0.32
N (% NPN)	5	6	5.6	6	9	10	6
hemolymph	17.2	28.5	29.8+	32.0+	26.7	32.0+	16.2*++
(mg/100 ml)	± 0.8	± 0.74	± 0.3	± 0.18	± 0.71	± 0.3	± 0.5
N (% NPN)	3	12	11.5	13	11	10.5	9
hypodermis	0.332	0.513+	0.545+	0.636	0.58	0.761	0.446
(mg/g)	± 0.03	± 0.8	± 0.032	± 0.60	±0.04+	±0.15+	0.05+++
N (% NPN)	1.5	2.6	2.6	3.2	2.8	3.6	6.3
gill (mg/g(	0.14	0.245	0.325	0.476	0.632	1.2	0.418
N (% NPN)	2.1	3.2	3.2	4.5	5.6	9.5	3.8
rate of	0.79	5.5	4.7	5.14	3.85	4.9	1.22
elimination (mg/animal/day)	± 0.25	± 2.62	± 1.83+	± 1.98+	± 1.42+	± 3.2+	±0.56++
N (% of NPN)	5.5	9.6	8.3	9.7	8.5	8.7	7.0

* Mean value within confidence interval for control.

+ > controls ++ < saline - injected control (SAL

remained high for 7 days afterwards. Injection of ESE has resulted in a decrease below SAL's level, but not down to the unoperated control's level.

Uric acid

Table 8 shows that uric acid made up a larger proportion in the hemolymph (5-10% of total NPN) than in the excreta. Plasma concentration has increased slightly but not significantly after ESX. ESE had no significant effect of concentration of uric acid of the hemolymph. Urin acid level has exceeded that of unoperated tissues examined except on day 2 post-operative. Concentrations of uric acid in the solid tissues were greater than in the hemolymph, percentage uric acid to total non-protein nitrogen was noticed to be higher in the tissues, reaching about 20% for the hypodermis. Uric acid represents an even small component (1%) of eliminated non protein-nitrogen as compared with urea nitrogen (Table 7).

Ammonia (NH3)

The results presented in Table (9) show that (NH_3) has increased in hypodermis only on days 1^{st} and 2^{nd} and in muscles on days 5th and 7th for gills. Only ammonia level was consistently higher than unoperated control's level during the entire period (7 days). In all other tissues, except hepatopancreas, ESE was followed by a decrease in ammonia below SAL's concentration.

The increased rate of elimination after ESX is not a simple consequence of ammonia increase in hemolymph. The ESE has decreased hemolymph ammonia below SAL's level but, within the range of confidence limits (confidence interval) for unoperated control for the other tissues.

Ammonia nitrogen made up 55 to 90% of NPN eliminated; the trend of variation in ammonia elimination rate is accordingly parallel to the of NPN. This rate has increased to about five-folds after ESX and then remained at that level afterwards. Injection of ESE on day 7th has decreased the rate below that of SAL's level but not to that of control's. The changes in ammonia hemolymph are to some extent, parallel to those of the rate of elimination.

Table (8): Relationship between the effect of eyestalk ablation and injection with extract of eyestalk on uric acid concentration in different tissues of ***Portunus pelagicus*** (L).

Tissue	*days after extirpation*						
	Control 0	1	2	3	5	SAL 7	ESE 7
Muscles	1.3	1.76	1.99	2.16	2.57	2.76	2.16
(mg/g ± S.D)	± 0.31	± 0.53	± 0.15^{+}	± 0.12^{+}	± 0.41^{+}	± 0.41+	± 0.41^{+}
N(% of NPN)	9.1	13	14	14	16	15.2	20
hep mg/g	10	15	14	12	13	14	9
hemolpmph	2.5	4.21	3.15	3.74	3.79	5.19	3.84
(mg/100 ml)	± 0.6	± 0.51	± 0.51	± 0.61	± 0.51	± 0.72	± 0.35
N(% of NPN)	5.3	9.2	6.4	8.0	8.3	10.5	11.0
hypodermis	2.6	4.21	4.1	3.76	3.42	3.74	2.85
(mg/g)	± 1.1	± 0.17^{+}	0.3^{+}	0.35	± 0.71*	± 0.36	± 0.41^{*+}
N (% of NPN)	35	45	41	41	41	36	50
gills (mg/g)	0.621	1.5	0.935	0.919	0.887	1.63	0.968
N (% of NPN)	18	32	17.3	15.2	15.1	25.9	15
Rate of elimination	4.24	26.65	27.53	25.87	22.36	28.28	8.4
(mg/animal/day)	± 1.5	± 5.1	± 5.3+	± 2.1+	± 6.31+	± 1.5+	± 1.32++
N (% of NPN)	56	79.2	84	86	83	88.8	75.8

* Mean value within confidence interval of control
+ > unoperated controls ++ < saline - injected control
hep = hepatopanecreas (SAL)

Table (9): Effect of extirpation and syestalk hormonal extract on ammonia concentration in different tissues of ***Portunus pelagicus*** (L).

Tissue	*days after extirpation*						
	Control 0	1	2	3	5	SAL 7	ESE 7
Muscles	0.583	0.595	0.658	0.638	0.98	1.62	0.548
(mg/g)	± 0.27	± 0.19*	± 0.12+	± 0.12+	± 0.12+	0.213 +	±0.18*+
N (% of NPN)	5.1	6.6	9.1	8.0	12.0	14.0	4.8
hepatopancreas	1.18	1.29	1.35	0.98	1.15	0.81	0.86
(mg/g)	± 0.18	± 0.53	0.15	± 0.13*	0.15*	± 0.22	± 0.12
N (% of NPN)	19	21	22	14	16	12	10
hemolymph	7.39	10.43	14.92	8.82	10.41	11.42	7.46
(mg/100 ml)	± 2.32	± 1.15	± 2.52	± 1.15*	0.59	± 2.48	±1.01*+
N (% of NPN)	50	68	88	20	65	58	57
hypodermis	2.6	4.20	4.1	3.78	3.42	3.75	2.81
(mg/g)	± 1.07	± 0.15+	± 0.25+	0.25	0.65*	± 3.1	0.52*+
N (% of NPN)	30	41	38	38	37	36	50
gills	0.612	1.4	0.962	0.92	0.87	1.61	0.86
(mg/g)	± 0.125	± 0.4+	0.151+	± 0.07+	± 0.06	± 0.3+	± 0.2+
N (% of NPN)	17	31	16.8	14.3	14.2	24.6	141.1
Rate of elimination	5.03	26.67	27.45	25.58	21.73	28.85	8.2
(mg/animal/day)	± 1.3	± 5.3	± 5.31+	± 2.1+	± 6.1+	± 1.4+	± 1.2++
N (% of NPN)	55.1	80.3	85.6	87.2	84	90.3	75.1

* Mean value within confidence interval of control

+ > controls ++ < saline - injected control (SAL)

DISCUSSION

In tyhe present investigation, the mean concentration of protein in the hemolymph was found to increase after eyestalk removal, and reached values statistically different from that of unoperated control only on day 5 post-operative, whereas protein concentration in gills was less than that of the control on day 3rd. For RNA, there was either a decrease in both hepatopancreas and gills or at least no increase in any tissue after eyestalk ablation (SEX). These findings are in consistent with those of Fingerman ***et al*** (1967) and Gorell and Gibert (1971), who reported an increase in RNA concentration after ESX in the hepatopancreas of the crayfish P***rocambarus clarkii.*** However, Nagabushanam and Diwan (1974) reported the disappearance of RNA from the tissues of ***Barytelphusa cunicularis*** after ESX. Raghavaiah, ***et al.***, (1980) demonstrated that RNA increases in the land crab ***Oziotelphusa senex*** Fabricius during the premoults stage, at which the Y-organ becomes active. The discrepancies noted then may be resulted from differences in the timing after ESX, which is limited as a period usually longer than 7 days. Hormones released by Y-organ at intermoult stage are inhibited by an eyestalk principle. Our results support the inference that an eyestalk factor, normally secreted during intermoutl-maintains levels of RNA in the tissues and insofar as this is an index of protein synthesis, also maintains this at the intermoult level. The ESX results in an immediate decrease in both RNA and protein levels in the solid tissues were examined.

The only change noted for DNA concentration was a gradual increase in hemolymph from day 3rd after ESX, to day 7th and was decreased by ESE. Raghavalah ***et al.*** (1980) noted an increase of DNA above intermoult levels in hemolymph of a land cran ***Oziotelphusa senex*** during premoult. Therefore, it is suggested that this change could be a result of mobilization of hemocytes after ESX, probably as a non-specific effect of injury. The fact that no increase in RNA of hemolymph was observed by this operation seems inconsistent, and our hypothesis should be tested independently. No significant variation in DNA percent was detected in other tissues, suggesting that neither resorption nor growth of tissues, (which are normally happened during premoult), had occured during the 7 days period of our experiment.

Amino acids, which have an important role in osmotic regulation in crustaceans (Schoefeniels and gilles, 1970), constitute a major fraction of NPN of all the tissues examined particularly, muscles. In accordance with earlier studies concerning deamination (as a major source of NH_3), amino acids concentration decreased immediately and continue decreasing after ESX, in hepatopancreas, muscles and hypodermis, and immediately but not continuouly in gills. Therefore, a decrease in whole-body amino acids nitrogen which however, was not sufficient to account either for the increase in protein N ot for the much larger increase in NH_3 nitrogen eliminated. The decrease in amino acid N on the tissues were reversed partly (muscles) or completelt (hepatopancreas and hypodermis) by ESE, and the concentration in hemolymph, which increased after eyestalk extirpation, was not affected by ESE. Raghaviah ***et al.*** (1980) reported an increase in concentration of free amino acids in the land crab ***Oziotelphusa senex*** after ESX, but this was probably a consequence of activation of the Y-organ, since it was reversed by crustecdysone, the hormone of that organ, No amino acid N was detected in the excreta, of ***Portunus pelagicus*** although it occurs frequently in other crustaceans (Ramamurthi, ***et al.*** 1982).

There is a significant increase in the elimination of NPN in all the days after ESX than that of unoperated control. Injection of ESE is followed immediately (12 hr) by a significant decrease in elimination rate. In other freshwater crustaceans, the rate of flow or urine is increased by ESX (Kamemoto and Ono, 1969) but the effect od SEX on the elimination of NPN connot be explained solely in terms of increased production of urine. The ratio of rate of elimination of NPN to the concentration of NPN in hemolynph (Proportional to urine: plasma ratio) was about 0.6 in control, and increased to about 2 in 3 days. At the same period, the ratio of NH_3, the most abundant component of elimiated N, increased from 0.6 to more than 3. Rations for minor components were generally smaller. In the present work, the increase in elimination NPN rate and of individual components there may be resulted from an increased production of the end-products of increasing catabolism rate. Raghavaiah ***et al.***, (1980) pointed out that the eyestalks of ***Oziotelphusa senex*** normally secrete, one or more factors inhibiting the catabolism of nitrogenous components of the different tissues.

The increase in the rate of elimination of NPN is largely, but no entirely, accounted for by the classical end-products; NH_3 urea and uric acid. The undetermined fraction of NPN, characteristic of crustacean excreta in general (Ramamuthi, ***et al.***, 1982); decreases after eyestalk extirpation and is returned back to control's level by injection of eyestalk extracts.

Variation in the rate of elimination of urea and uric acid, which constitute, respectively 5 and 1% of total NPN eliminated in unoperated controls was noted. These changes wre parallel to those of NH_3 except that elimination of uric acid, though significantly increased by ESE, was not thereby neturned to control levels. Tissue concentrations of these two substances have generally increased after ESX and then decreased after ESE, but the increase in (Urea) was immediate and continuous only in hypodermis, with some delayed increase in the other tissues after ESX. The ESE reversed the increase completely in hemolymph, muscles, and gills only partly in hypodermis, and not at all in hepatopancreas. Urea is probably derived only from hydrolysis of the amino group of agrinine by arginase since; arginine phosphate is known to be the phosphagen of crustacean muscles. Arginine is not known to be synthesized through the ornithine cycle in Crustaceans (Schoffeniels and Gilles, 1970). Uric acid is derived from oxidtion of purines set free by breakdown of nucleic acids and nucleotides. Purines and uric acid, are not known to be synthesized as part of an excretory pathway for N in crustaceans.

Although uric acid is a minor componentb(1&) of eliminated N, it is more important component of the NPN of tissues (10-20%). The features of uric acid metabolism noted here are of interest in relation to the known role of this substance as the principal endproduct of nitrogen metabolism in terrestrial arthropods.

Of the three known end-products eliminated, ammonia is the chief component as in aquatic animals in general (Ramamurth, ***et al.***, 1982). The rate of elimation of ammonia increases significantly after ESX and is restored immediately to control level by injection of ESE. Most of the NH_3 eliminated is probably derived from deamination of amino acids and from hydrolysis of the amide groups of glutamine and asparagine. It can be concluded that the eyestalk principle acts to inhibit these processes. The increased rate of elimination of NH_3 after ESX is only partly reflected in its concentration within

the tissues, but ESE results in significant decrease in ammonia in all tissues except hepatopancreas. The results of the present investigation agree with that of young-Lai ***et al.*** (1991) and Hui-peng Lin ***et al.***, (1993), for ***Homarus americanus***. They reported that the changes NH_3 in the gills closely reflect those in elimination, as in other aquatic animals, the gills serve as the main route of elimination of NH_3. Possibly, they may also be important sites for production of NH_3.

REFERENCES

Adiyodi, K.G., and Adiyodi, R.G., 1970. Endocrine control of reproduction in decapod crustacea. Biol. Rev., (45): 121-165.

Abd El-Hamid N.F., 1989. Metabolic effects of eyestalk ablation on the total protein and lipid of the prawn ***Penaeus Kerathurus*** (Leach). Bull. Nat. Inst. Oceanogr. & Fish, ARE, 15 (2): 129-141.

Abd El-Hamid N.F., 1994. Neuroeudocrine control of the hepatopancreas in the crab ***Portunus pelagics*** (Linnaeus). International conference on future Aquatic Resources in Arab region. : 283-291.

Chu, K.H. and Chow, W.K., 1992. Effects of unilateral versus bilateral eyestalk ablation on moulting and growth of the shrimp *Penaeus chinensis* (Osbeck, 1965) (Decapoda Penaeidea) Crustacean, 62 (3): 225-233.

Fingerman, M.T.; Dominczak M., Miyawaki C., Oguro C. & Yamamoto Y., 1967. Neuroendocrine control of the hepatopancreas in they crayfish ***Procambarus clarkii. Physiol Zool.***, 40-23-30.

Foster, L.B. and Hocholzer; J.M., 1971. "determination of urea nitrogen clin chem. 17, 1971.

Giles K.W., and Myers E., 1965. An improved diphenylamine method for estimation of deoxyribouncleic acid. Nature, Lond. 206: 93-97.

Glick D., 1966. Methods of Biochemical Analysis. Wiley, New York.

Gorell T.A. and Gilbert L.I., 1971. Protein and RNA synthesis in premolt crayfish ***Croconectes virilis***. Z. tergl. physiol., 73: 345-356.

Hashem, H.O., Abdel Hamid, N.F. and El-Sawi, N.M., 1991. "Histological studies on the ovaries of ***Penaeus kerathurus*** (Leach) after deprivation of the eyestalk hormones. J. Egypt. Ger. Soc. Zool. Vol. (5): 137-151.

Honke L.A. and Scheer, B.T., 1970. Carbohydrate metabolism in crustaceans. Chem. Zool. 5A. 147-166.

Hui peng Lin, Thuer P., Trilles J.P., Mounet Guillaume R., and Charmantier G., 1993. Effects of ammonia on survival and osmorgelation of various development stages of the shrimp ***Penaeus japonicus. Marine biology***, 117: 591-598.

Kamemoto F.I. and Ono J.K., 1969. Neuroendocrine regulation of salt and water balance in the crayfish ***Procambarus clarkii***. Comp. Biochem. Physiol. 29: 393-401.

McWhinnie M.A. and Mohrherr C.J., 1970. Influence of eyestalk factors, intermolt cycle, and season upon (C)14 leucine incorporation into protein in the crayfish ***Orconectes ririlis***. Comp. Biochem. Physiol, 34: 415-437.

Moore S. and Stein W.H., 1968. A modified minhydrin reagent for phtometric determination of amino acids and related compounds. J. Biol. Chem., 243: 6281-6283.

Nagabushanam, R. and Diwan, A.D., 1974. Neuroendocrine control of the hepatopancreas in the freashwater crab ***Barytelphusa cunicularis***. J. Anim. Norph Physiol. 21: 35-43.

Oer, B.L., 1965. Hawks physiological chemistry. McGraw-Hill, New York.

Raghaviah, K., Ramamurthi, R., Chandrasekharam, V. and Bradley, T.S., 1980. Neuroendocrine control of nitrogen metabalism in the Indian Field ***crab Oziotelphusa S. senex*** Fabricius 1-End- Products and elimination. Comp. ***Biochem. Physiol***. 67 (B): 437-445.

Ramamurthi, R., Raghavaiah, K., Chandrasekharam, V & Scheer, B.T., 1982. Neuroendocrine control of nitrogen metabolism in the Indian field crab ***Oziotelphusa S. senex***. Fabricuis comparative Biochemistry and physiology B 71: 223-228.

Renaud L., 1949. Le cycle des reserves organiques chez les crustaces decapodes. ***Annls Inst. Oceanogr***., Paris, 24: 259-357.

Scheer B.T., 1979. mammalian endocrines, Chem. Zool. 11: 103-158.

Scheer B.T. and Langford R.W., 1976. Endocrine effects, on the cation dependent atpases of the gills of eels Gen. Comp. Endocr, 30: 313-326.

Schoffeniels E. and Gilles R., 1970. Nitrogen constitutents and nitrogen metabolism in arthropods. Chem. Zool., 5A: 199-228.

Silverthorn, S.U., 1976. Hormonal involvement in the fiddler crab ***Uca pigilator***, Comp. Biochem. Physiol, 45A; 417-428.

Tsuyoshi, S.O. and Jaws, K.B., 1978. A simplified of quantitating protein using the biuret and phenol reagent. ***Anal. biochem***., 86: 193-200.

Van Harreveld, A., 1936. A physiological solution for freshwater crustaneans. Proc. Soc. Exo. Biol. Med., 34: 428-432.

Yamaoka, L.H. & Scheer, B.T., 1970. Chemistry of growth and development in crustaceans. Chem. Zool. 5A: 321-342.

Young- Lai, W.W., Charmantier- Daurces M. and Charamantier, G., 1991. Effect of ammonia on survival and osmoregulation in different life stages of the lobster ***Homarus americanus***. Mar. Biol. 110: 293-300.