Bull. Environ. Contam. Toxicol. (1998) 60:22-29

Bulletin of Environmental Contamination and Toxicology

Influence of Naphthalene on Protein, Carbohydrate, and Phosphatases System During the Vitellogenesis in Marine Edible Crab, *Scylla serata*

M. Elumalai,[1] S. E. Balasubramanian,[2] M. P. Balasubramanian[1]

[1]Department of Pharmacology and Environmental Toxicology, Dr. ALM. P. G. Institute of Basic Medical Sciences, University of Madras, Taramani 600 113, Chennai, Tamil Nadu, India
[2]Department of Zoology, Ethiraj College, Chennai 600 105, India

Received: 7 August 1997/Accepted: 20 October 1997

Although a number of workers have studied the biochemical components of the tissues of crustaceans in relation to pollutant stress (Murti *et al.* 1984 :Omkar 1985: Elumalai *et al.* 1977), no detailed information is available regarding the enzyme changes during the ovarian development of crustaceans in response to chemical stress, except the work of Victor and Sundar raj (1988). Reproduction is the main energy demanding physiological process. Several workers have observed definite fluctuation in the hepatopancreatic reserves of crustaceans correlated to ovarian maturation (Anilkumar and Adiyodi 1980: Balasubramanian and Subramoniam 1987). However an understanding of the enzyme systems that attend to the synthesis and breakdown of the substrate in the hepatopancreas, their mobilization via haemolymph and their resynthesis (or) direct accumulation in the ovary is meagre.

It is, therefore, of interest to study changes in protein and carbohydrate in the hepatopancreas and ovary of marine edible crab, *Scylla serrata* with reference to pollutant stress. Also to assess the quantitative differences of phosphatases with reference to vitellogenesis since these enzymes play a significant role in carbohydrate metabolism (Goodman and Rothstein 1957). Naphthalene is one of the prominant diaromatic fractions of crude and refined oils and it is a polycyclic aromatic hydrocarbon (PAH). Generally PAHs are less sensitive to photooxidation and therefore are more persistent in water (Rand and Petrocelli 1985). PAHs are rapidly accumulated by aquatic organisms reaching levels higher than those in the ambient medium and affect normal function of the aquatic life (Kulkarni and Masurekar 1984). Hence, naphthalene was selected as a toxicant in the present investigation.

MATERIALS AND METHODS

Female Scylla *serrata were* collected from Pulicate lake near Chennai, Tamil Nadu. They were acclimated to the laboratory temperature (28 ± l°C) in large glass aquaria

Correspondence to: M. Elumalai

for one week, adjusting the level of water to keep them just submerged. The water was changed daily. They were fed with flesh fresh prawns and divided into two groups. Crabs belonging to group I were reared in naphthalene free seawater and treated as a control. Crabs belonging to group II were exposed to naphthalene at 0.020 mg/L concentration. The treatments were continued up to 96 hr. After the exposure to naphthalene, the ovarian stages were classified based on the criteria explained by Balasubramanian and Subramoniam (1987). The hepatopancreas and ovary were separated from the exposed and control crabs. The total carbohydrate was determined by the method of Roe (1955), using glucose as standard. Acid and alkaline phosphatases were estimated as described by Balasubramanian *et al.* (1983), using p-nitrophenyl phosphate as substrate. The enzyme activities were expressed as mg p-nitrophenol/mg protein/hr. Protein was estimated according to the method of Lowry et al. (1951), using bovine serum albumin as standard. Each experiment was replicated five times and the data were subjected to Student's 't' test (Zar 1984).

RESULTS AND DISCUSSION

The effect of naphthalene on protein and carbohydrate level of ovary and hepatopancreas during vitellogenesis of *S. serrata* is presented in Table 1. Protein content of the ovary and hepatopancreas of control crabs increased from stage I to V. On the otherhand in stage II and IV there were reduction in the protein content. It is evident from the present investigation that the protein content of control crabs between stage IV and V indicates intense vitellogenic activity during this period as suggested by Balasubramanian and Subramoniam (1987). A decline in the organic reserves in stage II may therefore be due to heavy water intake occurring in the freshmoult stage. In the present investigation, decrease in the protein content in the hepatopancreas and the ovary are observed in naphthalene exposed *S. serrata.* The reduced content under chemical stress may be due to the utilization of amino acids in various catabolic reactions. The amino acids through transamination and deamination reactions might have supplied necessary ketoacids to acts as precursors for the maintenance of carbohydrate metabolism to meet the energy requirements during chemical stress condition (Jha 1991). The loss of protein under naphthalene stress noticed in the present study may be due to the reduced synthesis of proteins or to the inhibition of the enzymes involved in protein synthesis.

The carbohydrate content of ovary of the control crab was high only during the initial stages of vitellogenesis. This high content gradually dropped during vitellogenesis. In hepatopancreas of control crab, the total carbohydrate

Table 1. Effects of naphthalene on carbohydrate and protein level of hepatopancreas and ovary of *Scylla serrata* in various stages of ovarian maturation (C = Control; T = Treated)

stages		Carbohydrate (mg/lOO mg wet tissue)		Protein (mg/lOO mg wet tissue)	
		Hepatopancreas	Ovary	Hepatopancreas	Ovary
I	C	4.82 ± 1.18 (100)	4.55 ± 1.27 (100)	8.03 ± 1.16 (100)	8.56 ± 1.67 (100)
	T	2.40 ± 0.31** (49.79)	1.96 ± 0.68** (43.07)	5.23 ± 1.00** (65.13)	5.44 ± 1.13** (63.55)
II	C	6.91 ± 1.63 (100)	6.00 ± 1.44 (100)	6.89 ± 1.25 (100)	6.05 ± 0.82 (100)
	T	3.58 ± 0.84** (51.80)	3.53 ± 0.86* (58.83)	3.67 ± 0.90** (53.26)	3.58 ± 0.82** (59.17)
III	C	7.18 ± 1.44 (100)	5.19 ± 1.78 (100)	7.69 ± 1.24 (100)	8.23 ± 0.91 (100)
	T	4.00 ± 0.80** (55.71)	2.90 ± 0.68* (55.87)	3.99 ± 0.75*** (51.88)	4.85 ± 1.023*** (58.93)
IV	C	4.30 ± 0.82 (100)	3.73 ± 1.45 (100)	7.44 ± 1.01 (100)	6.38 ± 0.90 (100)
	T	2.01 ± 0.66** (46.74)	1.17 ± 0.63** (31.36)	3.37 ± 0.61*** (45.29)	3.68 ± 0.38*** (57.68)
V	C	8.09 ± 1.45 (100)	2.80 ± 0.71 (100)	8.31 ± 0.83 (100)	9.93 ± 1.67 (100)
	T	4.26 ± 1.33** (52.65)	0.85 ± 0.078*** (30.35)	5.30 ± 0.92*** (63.77)	5.49 ± 0.91*** (55.28)
VI	C	3.35 ± 0.80 (100)	1.41 ± 0.57 (100)	6.85 ± 0.94 (100)	7.61 ± 0.94 (100)
	T	1.47 ± 0.54** (43.88)	0.61 ± 0.078* (43.26)	2.69 ± 1.031*** (39.27)	4.57 ± 1.11** (60.05)

Values are expressed in means ± SD of 5 observations. Values in paraentheses are percentage change from control (100%). Asterisks indicate values which are significantly different from controls (Student's 't' test; Zar 1984).

*P<0.05, ** P < 0.01, *** P < 0.001

was high at the stage V of ovarian maturation. Which was declined in the spent stage VI. Carbohydrate content of control crab was high only during vitellogenesis. This was suddenly dropped at stage VI (spent). In the present investigation a decrease in carbohydrate content in hepatopancreas and ovary was observed in naphthalene exposed *S. serrata.* Dhavale and Masurekar (1986) state that decreased level of carbohydrate in tissues of toxicant exposed animals suggest a typical stress response confirming the prevalence of hypoxic condition at the tissue level, since anoxia or hypoxia increases carbohydrate consumption (Dezwaan and Zandee 1972), thereby creating a sort of stress on animals even at sublethal level resulting in extra expenditure of energy (Keller and Andrew 1973).

The effect of naphthalene on acid and alkaline phosphatase activity of the hepatopancreas and ovary of *S. serrata* in various stages of ovarian maturation is presented in Table 2 and 3. In the ovary of control crabs, acid phosphatase activity was high only in initial stages of vitellogenesis. The high activity declined in the spent stage VI. However, the ovarian alkaline phosphatase activity was high only in stage II and IV. which declined in spent stage VI. The hepatopancreas acid and alkaline phosphatases activity gradually increased upto IV stage and then declined in the spent stage VI. Acid phosphatase activity was always maintained at a higher level than that of alkalinephosphatase and that the hepatopancreas was rich in total acid and alkaline phosphatases activity. High ovarian acid phosphatase activity in stage II and IV was related to oogonial proliferation and active vitellogenesis. A steep increase in phosphatases activity in the hepatopancreas during stage IV was related to the hydrolysis of organic reserves during vitellogenesis. In the present investigation a rise in acid phosphatase activity in hepatopancreas and ovary observed in naphthalene exposed *S. serrata.* It may be recalled that acid phosphatase has been established as a marker for lysosomes thus, it is logical that the enzyme is hydrolytic in its function and acts as one of the several acid hydrolases in the autolysis process of the cell after its death (Novikoff, 1961). The increased acid phosphatase activity in the female *S. serrata* may be due to the toxic effect of naphthalene by which the cellular and lysosomal membranes might have been ruptured (Murti *et al.* 1984). In the present study a decline in alkaline phosphatase activity in the hepatopancreas and ovary is observed in naphthalene exposed *S. serrata.* This is in accordance with the findings of Elumalai *et al.* (1997). The decrease in phosphatase activity may be due to the uncoupling of phosphorylation by the naphthalene stress. It is well known that alkaline phosphatases are known to be involved in carbohydrate metabolism, growth and differentiation,

Table 2. Effects of naphthalene on acid phosphatase activity of hepatopancreas and ovary of *Scylla serrata* in various stages of ovarian maturation (C = Control; T = Treated)

Stages		Acid Phosphatase (mg P-nitrophenol/mg protein/hr)	
		Hepatopancreas	Ovary
I	C	0.0758 ± 0.0093 (100)	0.0240 ± 0.0080 (100)
	T	0.157 ± 0.047** (207.12)	0.0497 ± 0.0085** (207.08)
II	C	0.0972 ± 0.017 (100)	0.0378 ± 0.0088 (100)
	T	0.163 ± 0.045* (167.69)	0.0652 ± 0.010** (172.48)
III	C	0.176 ± 0.039 (100)	0.0175 ± 0.0037 (100)
	T	0.281 ± 0.078* (159.65)	0.0401 ± 0.0080*** (229.14)
IV	C	0.343 ± 0.059 (100)	0.0326 ± 0.010 (100)
	T	0.592 ± 0.054*** (172.59)	0.054 ± 0.011* (165.64)
V	C	0.225 ± 0.072 (100)	0.0192 ± 0.0051 (100)
	T	0,423 ± 0.030*** (188)	0.0411 ± 0.010** (214.06)
VI	C	0.190 ± 0.0094 (100)	0.015 ± 0.0042 (100)
	T	0.285 ± 0.039*** (150)	0.0320 ± 0.0098** (213.33)

Values are expressed in means ± SD of 5 observations. Values in paraentheses are percentage change from control (100%). Asterisks indicate values which are significantly different from controls (Student's 't' test; Zar 1984).

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table 3. Effects of naphthalene on alkaline phosphatase activity of hepatopancreas and ovary of *Scylla serrata* in various stages of ovarian maturation (C = Control; T = Treated)

Stages		Alkaline Phosphatase (mg P-nitrophenol/mg protein/hr)	
		Hepatopancreas	Ovary
I	C	0.0812 ± 0.010 (100)	0.0454 ± 0.0094 (100)
	T	0.0358 ± 0.0058*** (44.08)	0.025 ± 0.0049** (55.06)
II	C	0.100 ± 0.013 (100)	0.104 ± 0.015 (100)
	T	0.0538 ± 0.0081*** (53.8)	0.0316 ± 0.0069*** (30.38)
III	C	0.151 ± 0.026 (100)	0.0424 ± 0.012 (100)
	T	0.0606 ± 0076*** (40.13)	0.0135 ± 0.0011** (31.83)
IV	C	0.267 ± 0.046 (100)	0.182 ± 0.038 (100)
	T	0.0784 ± 0.014*** (29.36)	0.0834 ± 0.018*** (45.82)
V	C	0.162 ± 0.032 (100)	0.0539 ± 0.010 (100)
	T	0.076 ± 0.013*** (46.91)	0.0211 ± 0.0051*** (39.14)
VI	C	0.0972 ± 0.017 (100)	0.0268 ± 0.0078 (100)
	T	0.0406 ± 0.0066*** (41.76)	0.0126 ± 0.0011** (47.01)

Values are expressed in means ± SD of 5 observations. Values in paraentheses are percentage change from control (100%). Asterisks indicate values which are significantly different from controls (Student's 't' test; Zar 1984).

** P < 0.01, *** P < 0.001

protein synthesis, synthesis of certain enzymes, secretary activity and transport to phosphorylated intermediates across the membranes (Omkar 1985). The decline in alkaline phosphatase activity may be due to the disturbance in any one of above said processes.

In the present study high phosphatase activity in the hepatopancreas and a moderate amount in the ovary in *S. serrata* suggests that they may be involved in various metabolic events occurring simultaneously. The reduced levels of protein, carbohydrate and important enzymes of carbohydrate metabolism might be due to introduction of naphthalene into the circulatory system and interference with the function of female reproductive system. Hence it can be concluded that naphthalene affects the vitellogenesis of *S. serrata.*

Acknowledgements. We thank Prof.Dr.M.S. Krishnamoorthy Professor and Head, Department of Pharmacology and Environmental Toxicology, University of Madras, Chennai for the provision of facilities.

REFERENCES

Anilkumar G, Adiyodi KG (1980) Effect of eye-stalk ablation as hepatopancreatic reserves in *Paratelphusa hydrodromous.* All India workshop on crustacean reproduction, Calicut University. p27

Balasubramanian MP, Dhandayuthapani S, Nellaiappan K, Ramalingam K (1983) Comparative studies on phosphomonoesterase in helminths. Helminthologia 20: 111-120

Balasubramanian SE, Subramoniam T (1987) Biochemical fluctuations during ovarian development in the edible crab, *Scylla serrata* (Forskal). J Reprod Biol Comp Endocrinol 7 : 21-32

Dezwaan A, Zandee DI (1972) The utilisation of glycogen and accumulation of some intermediates during anaerobiosis in *mytilus edulis.* Comp Biochem Physiol 43 : 47-54.

Dhavale DM, Masurekar VB (1986) variations in the glucose and glycogen content in the tissues of *scylla serrata* (Forskal) under the influence of cadmium Toxicity. Geobios 13: 139-142

Elumalai M, Felista Rani E, Balasubramanian MP (1997) Effect of naphthalene on gonadal phosphatases and Esterases in marine Crab, *Scylla serrata.* Geobios 24 : 70-72

Goodman J, Rothstein A (1957) The active transport of phosphate into the yeast cell. J Gent Physiol 40: 915-925

Jha BS (1991) Alterations in the protein and lipid contents of intestine, liver and Gonads in the lead exposed freshwater murrel *Channa punctatus* (Bloch) . J Ecobiol 3: 29-34

Keller R, Andrew EM (1973) The site of action of the crustacean hyperglycemic hormone. Gen comp Endocrinol 20: 572-578

Kulkarni BG, Masurekar VB (1984) Effect of naphthalene exposure on blood serum enzyme activities in the crab *Scylla serrata* (Forskal). Indian J Mar Sci. 13: 97-98.

Lowry DH, Rosebrough MJ, Farr AL, Randall RJ (1951) protein measurement with the Folinphenol reagent. J Biol Chem 193: 265-275

Murti R, Omkar, Shukla GS (1984) mercuric chloride intoxication in freshwater prawn. II Effect on phosphatases activity. Ecotxicol Environ Saf 8: 581-586

Novikoff AB (1961) "The Cell" (Brachet J, Mirsky AF Eds) Academic Press, Newyork p423

Omkar (1985) changes in Acid and alkaline phosphatase activity of a freshwater prawn, *Macrobracium lamarrei* exposed to Aldrin. Ind Hlth 23: 155-157

Rand GM Petrocelli SR (1985) Fundamentals of aquatic Toxicology, Hemisphere Publishing Corporation, Washington

Roe JH (1955) The determination of Sugar in blood and spinal fluid with anthrone reagent. J Biol Chem 212: 335-343

Victor B, Sundar raj A (1988) changes in the developmental morphology of Oocytes in urea toxified crab, *Paratelphusa hydrodromous.* Proc of the fifth all India Symposium of Inverte reprod p219

Zar JH (1984) "Biostatistical analysis", 2nd Edition, Prentice Hall International Inc., Englewood Cliffe