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BIOCHEMICAL ESTIMATION OF GLYCOGEN LEVELS IN THE HAEMOCYTES OF FRESHWATER CRAB *BARYTELPHUSA CUNICULARIS*

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ABSTRACT

The haemocytes found in the blood of crustaceans are the main mediators of host defence against infections in crustaceans . Glucose is the principal monosaccharides present in the hemolymph of crustaceans .Glucose is stored in the form of glycogen .The stored glycogen is utilized in moulting ,adaptation to hypoxia and /or anoxia, osmoregulation and during fasting periods .The variation in glucose in the haemolympatic glucose seem to be related to the reproductive period of the species ,food availability and degree of environmental exploration .these factors led to different metabolic adjustment in distinct species of crustaceans. In this study the biochemical glycogen analysis was estimated in the haemocytes of both male and female freshwater crabs *Barytelphusa cunicularis*. The results showed that the average carbohydrate per cells in females is significantly more than that of males.

KEYWORDS:Acetylcholin.esterase, hemocytes,biochemical,glycogen,B.cunicularis.

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INTRODUCTION

Acetylcholine, as a neurotransmitter at synaptic junctions. This enzyme is used in experimental work(Ach E,EC, 3.1.1.7). It is a membrane bound enzyme which belongs to the hydrolases. Its localization in lymphocyte membrane was demonstrated by immunoflourescent method by is well established kutty et al [1] has suggested the functional role for acetylcholine and acetylcholinesterase, respectively at non nervous sites also. The metabolic role of Ach., has been reported[2].Besides the role as a neurotransmitter [3] suggested the role of Acetylcholine in respiration and is also found iblood cell membranes. Acetylcholinesterase, an enzyme has been found in thefree state mainly in nerve cells, lung, erythrocytes play an important role in transmitting of nerve impulses.[3] Acetylcholinesterase has been first purified from electric organ tissue of electric eel *Electrophorus.electricus* by conventional techniques as a soluble globular protein with a sedimentation coefficient of about 11S[4]. acetylcholinesterase is widely distributed in excitable membranes of nerve impulses, because of its involvement in nervous transmission, this enzyme catalyses the hydrolysis of Acetylcholinesterase with a relative specificity for Acetylcholine and is bound to cellular membranes of excitable tissue(synaptic function, endoplasmic etc) and is believed to be associated with nerve impulse conduction and also found in blood in the cells.[5 6 7] Acetylcholinesterase is widely distributed throughout the crustacean nervous system even in locations were Acetylcholine is not a neurotransmitter. It is well demonstrated in glial sheaths. It is likely the Acetylcholine released from the glial cells during nerve activity (due to potassium depolarity) and that a reaction with the receptors on the gial membrane, regulate the membrane potential of the glial cell by hyperpolarising it,[8].

MATERIAL AND METHODS

ESTIMATION OF CARBOHYDRATES

Total Carbohydrates was estimated using Anthrone reagent. [9]

Carbohydrates are an important component of storage and exist as free sugars and polysaccharides. The basic units of carbohydrates are the monosaccharide which cannot split by hydrolysis into more simple sugars. The carbohydrate content can be measured by hydrolyzing polysaccharides into simple sugars by acid hydrolysis and estimating the resultant monosaccharide.

1)ANTHRONE REAGENT
Dissolve 200mg Anthrone in 100 ml of ice cold 95% H2SO4. Prepare fresh before use.

2)STANDARD GLUCOSE SOLUTIN: a)STOCK STANDARD: Weigh 100 mg of Glucose and transfer it carefully into a 100 ml with distilled water(100 mg of Glucose in 100 mL Distilled water).

Working Standard :- Dilute 10 mL of stock standard solution in 100 mL with distilled water in a volumetric flask.(100µg/ml).
Weigh 100mg of sample, homogenize mix well with 50 ml of distilled water. Add 50 ml OF 5% TCA solution mix well. Centrifuge at 2500 rpm for 10 min and filter the supernatant .pipette out 0,5 and 1.o ml of supernatant into into different test tubes . Similarly pipette out 0.2,0.4,0.6,0.8 and 1.0 ml of glucose working standard solution into different test tubes and adjust the volume using 1ml distilled water. Pipette out 1ml distilled water in a separate tube to set a blank. Add 10 ml Anthrone reagent to each test tube and place the test tube in boiling bath for 10-15 min . Cool the test tubes at room temperature for 30 min . Determine the optical density at 630nm in a spectrophotometer. Draw a standard graph by plotting concentration of the standard on the X- axis versus absorbance on the Y-axis. From the graph calculate the amount of carbohydrate present in the sample.

STATISTICAL ANALYSIS

Biochemical test were carried out in the haemolymph pellet separately for both male and female crabs. The average carbohydrate per cell in females is significantly more than that of males(P< 0.05), Microgram percentage per cell per microliter showed a significantly higher average of carbohydrate in female crabs as compared to males.

Microgram percentage composition of carbohydrates, per cell in the haemolymph pellet of freshwater crab Barytelphusa cunicularis

Table 1

BIOCHEMICAL COMPOSITION	MALE	FEMALE
CARBOHYDRATE	0.27%	2.9%

Values are in X± SD=3., µg% / Cell.

Microgram percentage composition of carbohydrates, in cells per microliter in the haemolymph pellet of freshwater crab Barytelphusa cunicularis.

Table 2

BIOCHEMICAL COMPOSITION	MALE	FEMALE
CARBOHYDRATE	11.09%	20.5%

Values are in X± SD=3., µg% / µl.

DISCUSSION

Biochemical analysis were carried out in the haemocytes of fresh water crab *B.cunicularis* the carbohydrate content in microgram per cell and cells per micro liter were detected in the hemolymph of both male and female crabs an estimated content of microgram per cell showed a higher content of carbohydrate in male and female crabs is almost the same, there existed a significant difference in the composition of carbohydrate in cells per microliter showed a difference in carbohydrate. Similar results were detected in the hemolymph of many crustaceans species studied by.,Kucharski and Silva,1991a[10]. ,1991b [11] The variation of carbohydrate content in the crabs was due to the reproductive period of the species, food availability and environment as studied in Aegla ligulata (crustacean: Anomura: Aeglidae)by Olivera et al., 2007[12].

CONCLUSION

In this study of the biochemical analysis was estimated in thes hemocytes of both male and female freshwater crabs *B.cunicularis*. The variation of composition of carbohydrate in haemolymph of both the sex of the crab, the female crabs showed a slightly higher composition than males which may be due to the reproductive period of the species.

REFERENCES

1. Kutty KM, Chandra RK , .Shaktichandra. Acetylcholinesterase in erythrocytes and lymphocytes its contribution to cell membrane structure and function. Expermentia.1976Mar 15 ; 32(3):289-291.
2. Krnjevic K. Chemical nature of synaptic transmission in vertebrates. Physiol Rev. 1974 April 1,54(2):418-540.
3. Bodhke MK, Effect of some pesticidal pollutants on the physiology of Barytelphusa cunicularis. Ph.D thesis .Marathawada University,Aurangabad Maharashtra.1983.
4. Kremzner LT and Wilson IB. A chromatographic procedure for the purification of Acetylcholinesterase . J Biol chem. 1963 May; 238,1714-1717.
5. NachmansohnD : Proteins in excitable membranes. Science .1970Dec11; 170(3963):1228-1229.
6. Friedenberg M, and SeligmanA.Acetylcholinesterase at the myoneural junction: cytochemical ultrastructure and some biochemical considerations. J Histochem. Cytochem 1972 oct20(10):771-792.
7. PolitoffA, RoseS : Incorporation of acetylcholine into synaptic vesicles is associated with blockade of synaptic transmission.Nature.1975July24; 324-325.
8. Libeman EM, and SmileyKA . Electrophysiological and pharmacological properties of glial cells associated with the medial giant axon of the crayfish with implications four neuron-glial cell interactions. Ups J Med Sci.1980;85(3):331-342.
9. HodgeJE and HofreiterBT . Determination of reducing sugars and carbohydrates in. whistler R L and WolformML., Eds.Methods in carbohydrate chemistry. Academic press New York.1962; vol 1 380-394.
10. Kucharski LCR, and Da Silva RSM . Effects of diet composition on the carbohydrate and lipid metabolism in an esturine crab Chasmagnathus granulate (Dana, 1851). Comp Biochem. Physiol , 1991a ; 99A:215-218.
11. Kucharski LCR, and Da Silva RSM . Seasonal variation on the energy metabolism in an estuarine crab, Chasmagnathus granulata(Dana 1851). Comp.biochem . Physiol .1991b, 100A: 599-602.
12. Oliveira GT, Fernandes FA, Bond-BuckupG, Bueno AA, Silva RSM :- Seasonal variations in the intermediate metabolism of *Aegla ligulata* (Crustacea: Anomura:Aegiladae). Comp Biochem . Physiol. 2007; A147:600-606.