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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 defense against infections in crustaceans. Glucose is the principal monosaccharides present in the haemolymph 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(P< 0.5).

**KEYWORDS:** Acetylcholineesterase, hemocytes, biochemical, glycogen, B.cunicularis.

## INTRODUCTION

Acetylcholine, as a neurotransmitter at synaptic junctions is well established (kutty. et.al., 1976) has suggested the functional role for acetylcholine and acetylcholinesterase, respectively at non nervous sites also. The metabolic role of Ach, has been reported(Kvnjeric, 1974). Besides the role as a neurotransmitter (Bodhke, 1983) suggested the role of Acetylcholine in respiration and is also found in blood cell membranes.

Acetylcholinesterase, an enzyme has been found in free state mainly in nerve cells, lung, erythrocytes play an important role in transmitting of nerve impulses. 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 about11s (Kremzner, et.al., 1963).

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 (Nachmansohn,1970., Friedenberg and Seligman,1972., Politoff. et.al.1975).

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 (Mayanand, 1971b). 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, (Lieberman and smiley. 1979).

## MATERIAL AND METHODS

### Estimation of Acetylcholinesterase

Reagents: Buffered substrate

1) 0.1M NaCl.0.2M MgCl2,0.005% gelatine and 2.7×10 _-3 Acetylcholine in 0.02 M sodium phosphate, ph 7.0.
2) Acid: 4N Hydrochloric acid.
3) A lkaline Hydroxylamine: Prepare immediately before use by adding equal volumes of 2M Hydroxylamine and 3.5 N sodium hydroxide.
4) Ferric chloride:-0.37M in 0.1 N HCL.

**Method:** Haemolymph was from the chelicercae of the male crab with a syringe taking care not to clot and was centrifuged at 100× g for 3 minutes, the pellet containing the hemocyte sample was sucked in the hemocytometer tube until 0.5 mark and distilled water till 11 mark, it was then homogenized and centerfuged at 800×g for 20 minutes. The supernatant was discarded and to the resulting pellet was added an equal volumeof 30mM sodium(5.5ml) phosphate buffer and 1% of Triton ×100(0.05 ml in 100 ml distilled water) from which 0.5 ml was used and centrifuged at 100,000× g for one hour. The resulting supernatant was

diluted to 10 ml of 30 mM sodium phosphate buffer from which 0.5 ml of the diluted sample was used.

Into 1.0 ml of the buffered substrate was pipette 0.5 ml of the sample. After 2 minutes incubation at 25˚C, the reaction was stopped by the dilution of 2.0 ml of alkaline hydroxylamine. After an additional one minute 1.0 ml of acid was added followed by 1.0 ml of ferric chloride solution. The absorbance of the final solution was read at 540nm, against H2O. A blank was prepared in a manner excluding the enzyme and should fall within the optical density range 0.480-0.520.

**ESTIMATION OF CARBOHYDRATES**

Carbohydrates are important components 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.

**Carbohydrate Estimation By Anthrone Reagent**

(Hegde, JE and Hofreiter, 1962).

**Principle**

Carbohydrate are first hydrolysed into simple sugars using dilute hydrochloric acid. In hot acidic medium glucose is dehydrated to hydroxyl methl furfural. This compound forms with acetone a green coloured product with absorption maximum at 630nm.

**MATERIALS**

**1) Anthrone Reagent**

Dissove 200mg Anthrone in 100 ml of ice cold 95% H2SO4 prepare fresh before use.

**2) Standard Glucose Stock**

Dissolve 100 mg in 100 ml distilled water.

**Working standard:** 10 ml of stock diluted to 100 ml with distilled water.

**METHOD**

A series working standard glucose solution was pipette put into a series of tubes and the volume made to 1ml with distilled water 100µl of the haemocyte sample was added

separately in three tubes and made to 1ml with distilled water. The blank was set in a tube containing 1ml distilled water. To each of the standard and the sample tubes was added 4ml of freshly prepared Anthrone reagent and mixed properly in a cyclomixer, then heated for eight minutes in a boiling water bath, cooled rapidly and green to dark colour was read at an absorbance of 630nm. A calibrated plot of the standard carbohydrate was made and the concentration in the test sample was estimated by interpolation. The mean of the test samples was calculated. The µg of the carbohydrate was estimated using the standard graph.

## RESULTS

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, proteins and lipid per cell in the haemolymph pellet of freshwater crab *Barytelphusa cunicularis***

| Biochemical Composition | Male | Female |
|---|---|---|
| Carbohydrate | 0.27% | 2.9% |

Values are in X± SD=3., µg% / Cell.

**Microgram Percentage Composition of Carbohydrates, Proteins and Lipid In Cells Per Microliter In The Haemolymph Pellet Of Freshwater Crab *Barytelphusa Cunicularis*.**

| 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 (Oliveira et al., 2001b., Kucharski and Silva, 1991a).

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., 2003).

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