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Role of carbohydrate in male *Hydrophilious olivaceous* life processes

Sudhanshu Kumar*
Department of Zoology, Rajendra College, J. P. University, Chapra, Bihar, India

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Abstract: Carbohydrate metabolism in insects follows a more or less similar pattern as in vertebrates. In insects, like other animals, glucose has a central place in carbohydrate metabolism. But in majority of insect's species, the amount of free glucose is quite small. The most prominent sugar of haemolymph and other body tissues of insect is trehalose, a disaccharide of glucose. It has a glycocidic linkage between anomeric carbon atoms of both glucose molecules. It is a non reducing sugar. In most cases, the sugar contents in the haemolymph is relatively low level of glucose along with higher trehalose contents. In the present investigation on male *Hydrophilous olivaceous* it was observed that the copper sulphate had an initial carbohydrate lowering effect and latter elevating trend on the haemolymph, fat body, and testis of and male insects. Overall initial decline of the carbohydrate content in haemolymph, fat body, ovary and testes were observed in insects exposed to sub-lethal concentrations of copper sulphate.

Key words: Carbohydrate metabolism, *Hydrophilous olivaceous*, glucose, copper sulphate.

INTRODUCTION

The problem of pollution and its effect on cellular metabolism of an organism is so vital in present complex and civilized society that it has posed a serious threat to purposeful existence of humanity. It has been aptly remarked that during the last decade we have seen a marked increase in the use of toxicants, detergents, industrial effluents, petrochemical products, psychoactive drugs, productive insecticides and other chemicals in our society. It affects all metabolic processes including carbohydrate metabolism directly or indirectly.[1]

There is no doubt that carbohydrates are the most vital and versatile organic compounds of the living world. These are the most abundant among the major classes of bio-molecules. Chemically they are simple organic compounds that are aldehyde or ketone derivatives of the polyhydric alcohol or as compounds that yield these derivatives on hydrolysis. It is an important source of energy for vital activities. It has central role in cellular metabolism of all living organisms.

Carbohydrate metabolism in insects follows a more or less similar pattern as in vertebrates. In insects, like other animals, glucose has a central place in carbohydrate metabolism. But in majority of insect's species, the amount of free glucose is quite small. The most prominent sugar of haemolymph and other body tissues of insect is trehalose, a disaccharide of glucose. It has a glycocidic linkage between anomeric carbon atoms of both glucose molecules. It is a non reducing sugar. In most cases, the

*Corresponding author :
Phone :
E-mail : @gmail.com

sugar contents in the haemolymph is relatively low level of glucose along with higher trehalose contents.[1-3]

Metamorphic changes in both exopterygote and endopterygote are usually accompanied by substancial depletion of their carbohydrate reserves. During this period glycogen and trehalose supply glucose which provides an energy source and the substrate for various metabolic processes. Wyatt gave a comprehensive account of this process in insects.

In the present investigation on male *Hydrophilous olivaceous* it was observed that the copper sulphate had an initial carbohydrate lowering effect and latter elevating trend on the haemolymph, fat body, and testis of and male insects. Overall initial decline of the carbohydrate content in haemolymph, fat body, ovary and testes were observed in insects exposed to sub-lethal concentrations of copper sulphate.

MATERIALS AND METHODS

Quantitative estimation of haemolymph, fat body, ovary or testis carbohydrates of treated and control insects were done by Anthrone reagent Method.

The Anthrone reagent is the basis of a rapid and convenient method for the determination of hexose, aldopentose, hexuronic acid, either free or present in polysaccharides. The blue green solution shows absorption of maximum at 620 nm although some carbohydrates may give another colour.

Reagents

Anthrone reagent: 0.2 % in concentrated H_2SO_4.

Mixture of glucose: 10 mg / 100 ml and glycogen 10 mg / 100 ml (as standard).

TECHNIQUE

(A) Technique of Haemolymph Carbohydrates:-

Insect heads were pricked with a needle and were kept inverted in centrifuge tubes, one insect in one centrifuge tube and were centrifuged at 1500 rpm to collect haemolymph. 0.1 ml haemolymph was taken in separate centrifuge tube and added 5 ml TCA to precipitate protein. It was centrifuged at 3000 rpm for supernatant; test for carbohydrates was carried out.

(B) Technique of Fat body, Testis Carbohydrates

Treated and control insects were dissected. Fat body and ovaries or testes were taken out and crushed in 2 ml of distilled water with the help of mortar and pestle separately. The crushed material was collected in a centrifuge tube and 4 ml of 10% TCA was added to obtain the precipitate of proteins. It was centrifuged at the 3,000 rpm. The supernatant was kept separately for performing quantitative assay of carbohydrate.

4 ml of Anthrone reagent was added to 1 ml of test solution and has rapidly mixed. Boiled in water bath, the absorption was read at 620 nm against the anthrone reagent as blank.

Calculations

The carbohydrate content was calculated as follows:-

$$\frac{\text{OD of Unknown Solution}}{\text{OD of Known Solution}} \times \text{Concentration of Solution} \times \text{Dilution of Known Solution}$$

Where OD = Optical Density.

RESULTS AND DISCUSSION

Observation of carbohydrate content in *H. olivaceous.* The total carbohydrate content in the haemolymph of mature female and male *H. olivaceous* were 600 ± 10 SD mg/100 ml and 605 ± 9 SD mg/100 ml respectively in control insect.

Carbohydrate Content in Mature Male *H. olivaceous*

Haemolymph Carbohydrate Content

Almost similar carbohydrate lowering effect on the haemolymph carbohydrate content was observed in mature male *H. olivaceous* on treatment with copper sulphate. At 24 hrs of treatment the carbohydrate content declined to 185 ± 6 SD mg/100 ml of haemolymph. Similar declining pattern was observed at 48 and 72 hrs after copper sulphate treatment that was 165 ± 4 SD mg/100 ml and 160 ± 4 SD mg/100 ml, showing a rising trend, 350 ± 5 SD mg/100 ml after 168 hrs of treatment. All differences were highly significant as compared it with that of control insects, (Table - 1).

Fat body Carbohydrate Content

The mature male *H. olivaceous* had a total carbohydrate content of 60 ± 4 SD mg/gram of fat body in untreated condition or in control insects, (Table - 1).

The carbohydrate content in the fat body of mature male *H. olivaceous* after different periods of treatment with copper sulphate were 35 ± 3.5 SD mg/gram at 24 hrs, 25 ±

2.5 SD mg/gram at 48 hrs, 16 ± 1.5 SD mg/gram at 72 hrs and 48.5 ± 4 SD mg/gram at 168 hrs. All these differences were highly significant ($P < 0.001$) as compared it with that of control insects, (Table-1).

Testicular Carbohydrate Content

The carbohydrate content in the testis of mature male *H. olivaceous* was 65 ± 5 SD mg/gram in control insects, (Table - 1).

Table-2 Carbohydrate content in haemolymph, fat body and testes of mature male *H. olivaceous* during control and after different periods of treatment with Copper sulphate

Periods of Treatment	Haemolymph mg/100ml	Fatbody mg/gram	Testis mg/gram
Control	605 ± 9	60 ± 4	65 ± 5
24 hrs	185 ± 6***	35 ± 3.5***	17 ± 1.7
48 hrs	165 ± 4***	25 ± 2.5***	15 ± 1.5
72 hrs	160 ± 4***	16 ± 1.5***	12 ± 1
168 hrs	350 ± 5***	48.5 ± 4***	35 ± 2.5

Mean ± SD SD = Standard Deviation
*= $P < 0.05$ (Significant) ** = $P < 0.01$ (Significant)
*** = $P < 0.001$ (Significant)

Copper sulphate had also a declining effect on the testis carbohydrate content in the mature male *H. olivaceous*. Carbohydrate content were 17 ± 1.7 ($P < 0.001$) SD mg/gram, 15 ± 1.5 ($P < 0.001$) SD mg/gram, 12 ± 1 ($P < 0.001$) SD mg/gram and 35 ± 2.5 ($P < 0.001$) SD mg/gram of ovary at 24 hrs, 48 hrs, 72 hrs. and 168 hrs respectively after copper sulphate treatment, (Table - 1).

Carbohydrate is structural and functional component of all insect tissues. It is found in nuclei, cytoplasm, and cell membrane as well as in extracellular fluid and supporting tissues. Carbohydrate with proteins and lipids form the principal classes of organic compounds that are found in insect and other organisms.

Insects have chitinous exoskeleton which is rich in amino polysaccharides. In addition, insects differ from vertebrates as trehalose, a disaccharide acts as the storage form of glucose in them. The synthesis and hydrolysis of trehalose is a central hub of carbohydrate metabolism of insects. The main site for the synthesis of trehalose from glucose is the fat body.[1] In most insects, Carbohydrates are present as glycogen and trehalogen which can readily be converted into glucose. In addition, various amounts of glycoproteins may be present especially in the haemolymph.

Sugar content of whole insect have been studied in some cases; Egorova and Smolin described a steady rise in trehalose level from egg to pupal stage followed by a decline in the subsequent development to the adult insect in *Antheraea pernyi.* The absorption of glucose through gut wall, its conversion into trehalose in fat cells and finally its distribution in different body tissues were first demonstrated by Kilby using radio isotopes in locust.

In *Bombyx mori*, Nandi reported increased glycogen concentration in the whole body, fat body and gut during the early period of pupal life which then decreased up to the emergence of the adult from the cocoon. In the gonads, the increasing trend of glycogen continued up to the latter half of the pupal life particularly into ovary.

Marked sex wise variation in the carbohydrate level has been reported for *Tribolim castaneum and Coregon cephalonica*[2] *Sphaerodema rusticum*[4], *H. olivaceous*[1], *L. maculatus*. The influence of pesticides and chemicals on the carbohydrate metabolism has been studied in different animals including insects.

In the present investigation on male *Hydrophilous olivaceous* it was observed that the copper sulphate had an initial carbohydrate lowering effect and latter elevating trend on the haemolymph, fat body, and testis of male insects. Overall initial decline of the carbohydrate content in haemolymph, fat body, ovary and testes were observed in insects exposed to sub-lethal concentrations of copper sulphate.

The carbohydrate content in the haemolymph, fat body and gonads of 25 ± 2 SD mg/gram in testes in control insects. It was 20 ± 2 SD mg/gram in testes at 24 hrs of treatment.

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