CHAPTER - III

BIOCHEMICAL STUDY ON
Polypheretima elongata
INTRODUCTION

Different environmental factors are likely to affect biological systems in different ways according to their respective physico-chemical properties. Exploitation of natural resources by the human activity is altering the quality of an environment. In the world of biology the chemical element are arranged in complex pattern to form the body of animals. The combination of carbon, nitrogen, phosphorus, sulphur, oxygen, hydrogen and other element form carbohydrates, amino acids, peptides, proteins and other organic compounds. Animal body is composed of such substances, together with other materials such as water and mineral salts. The analysis of biological substance has been given a great impetus by the application of modern techniques such as chromatography, electrophoresis, flame photometry and electron microscopy. These methods have both simplified and expanded the task of these interested in the structure of the tissue of animal

Proteins are the most abundant macromolecules and constitute over half of the dry weight of most organisms. Proteins are extremely complex nitrogen containing molecules, which play important role in nearly all biological processes as structural components, biocatalysts, hormones and repositories of genetic information. They also help in storage, transport, mechanical support, control of growth and differentiation (Kale, 2002).

Carbohydrates play vital and central role in cellular biochemistry, in addition to their functions as structural units and food reserves (Rao and Murthy, 1980). Carbohydrate metabolism in the animal is to meet the energy demands by the organs and systems for proper functioning. In the animal the chief carbohydrate of the tissue is glycogen, while glucose is of the haemocoelomic (blood) and other body fluids (Holden, 1972).

Glycogen, a storage polysaccharide is reversibly converted to glucose. The equilibrium between the glycogen and glucose conversion tends to maintain blood glucose in a steady state. The equilibrium between glycogenesis and glycogenolysis is governed by the extrinsic and intrinsic environmental factor that governs the physiology of organs (Pickering et al., 1983).
Lipids are heterogeneous group of water insoluble (hydrophobic) organic molecule that can be extracted from tissues by non-polar (organic) solvents. Because of their insolubility in aqueous solution, body lipids are generally found either compartmentalized as in the case of membrane associated lipids and droplets of triacylglycerol in adipocytes or transported by plasma in association with protein as lipoprotein particles (Villalan et al. 1990). Lipids are not only a major source of energy but also provide the hydrophobic barrier that permits partitioning of the aqueous contents of cells and sub cellular structures. Lipids perform many functions in the body and imbalance of lipids metabolism can lead to severe problems (Amudhavalli et al., 1988).

The organisms respond to environmental stress by developing necessary potential to counter act that stress. The biochemical changes occurring in the body gives first indication of stress. During stress an organism needs sufficient energy, which is supplied from reserve materials i.e. protein, glycogen and lipids. If stress is mild then stored glycogen is used as a source of energy, but when stress is strong, then the energy stored in lipid and protein may also be used (Mark, 1969).

The information about biochemical composition of earthworms is extremely sparse. Lawerence and Miller (1945) and French et al. (1957) reported that proteins accounts for 53.5% to 71.5% of the dry weight of the earthworm body. Roch, (1979) analyzed the protein from coelomic fluid of *Eisenia fetida*. Roch, et al., (1984) Isolated agglutinins from lysins in the earthworm coelomic fluid by gel filtration followed by chromat focusing. Wojdani et al. (1982) have reported agglutinins and proteins in the earthworm, *Lumbricus terrestris*, before and after injection of erythrocytes, carbohydrates and other materials. Stein and Cooper (1983) have studied the carbohydrate and glycoprotein inhibitors of naturally occurring and induced agglutinins from the earthworm *Lumbricus terrestris*. Canicatti (1990) has studied the poreforming proteins in invertebrates. Eue et al., (1991) analysed the comparative characterization of lectins in the coelomic fluid of lumbricides. Roch et al. (1991) reported purification of three serine proteases from the coelomic cells of earthworms (*Eisenia foetida*). Lange
et al., (1997) have determined interaction of hemolysin of earthworm with erythrocyte and phospholipid membranes. Quaglino et al. (1996) and Cossarizza et al., (1996) studied earthworm leukocytes that are not phagocytic and cross react with several human epitopes killing human tumor cell lines.


Since quite fragmentary information is available regarding changes in the biochemical constituents and impact of environmental factors the present study has been undertaken with the aim to determine the effect of salinity (sublethal dose of sodium chloride) on the biochemical parameters like protein, glycogen and lipid in different tissue of the earthworm Polypheretima elongata.
MATERIAL AND METHODS

The earthworm *Polypheretima elongata* having approximately equal size and weight were collected from non-irrigated field, which had the record of heavy water logging in monsoon season. The soil had the following characteristics: laterite type, sandy loam texture, pH-6.8, organic matter 2.7 g%, nitrogen 0.22 g% and a C/N ratio of 12.27. The soil was air dried and sieved before use. The earthworms were acclimated for one month with adequate provision of food (10% organic matter, cow dung + leaf litter), moisture (20g%) and temperature (25±2°C). Earthworms were kept half immersed in glass petriplates containing 30ml of tap water at 25±2°C temperatures for 24 hours to evacuate their guts (Dash and Patra, 1977). The study was carried out in plastic culture pots under laboratory conditions. The worms were exposed to 24 h LD\textsubscript{50} sublethal dose (1.05g/kg soil) of sodium chloride for 5 days. The biochemical analysis like protein, glycogen and lipid were done by using the methods of Lowry *et al.* (1951), Dezwaan and Zandee, (1972) and Barnes *et al*., (1973) respectively. The changes were observed in skin, ovary, testis and intestine of worm *Polypheretima elongata*.

**Estimation of total proteins (Lowry *et al*., 1951):**

50-100 mg of tissue was homogenized in 5 ml cold distilled water. 5 ml of 30% TCA was immediately added to precipitate the protein. Precipitate was collected after centrifugation at 3000 rpm for 15 minutes. The pellet was repeatedly washed with distilled water to remove the traces of TCA. Precipitated protein was redissolved in 0.1N NAOH and estimated by the method of Lowry *et al.* (1951) as follows:

0.1ml of the solution was transferred into a test tube and 4 ml of alkaline copper sulphate reagent was added, followed by 0.4 ml of diluted commercial Folins reagent. The optical density of the blue colour developed was read at 540 µm after 30 minutes of addition of the Folins reagent using UV-VIS spectrophotometer (Model Digispec 200 GL). Bovine serum albumin was used as
a standard. The protein content was expressed as mg/100 mg wet weight of the tissue.

**Estimation of glycogen (DeZwaan and Zandee, 1972):**

Homogenate of 50 mg tissues was prepared of in 30% KOH and glycogen was estimated by the method of DeZwaan and Zandee (1972). The homogenate mixture was kept in boiling water bath for 3 to 5 minute to dissolve the tissue and then cooled. Before centrifugation 2 ml of 96% ethyl alcohol was added and the mixture was kept overnight in refrigerator. Next day this mixture was centrifuged at 3000 rpm for 15 minutes. The glycogen cake settled down on the bottom. 2 ml of distilled water was added to the cake and mixed well. This mixture was kept at 70°C for 5 minutes in hot water bath. 0.1 ml of the solution was mixed with 0.9 ml of distilled water and 5 ml of anthrone reagent was added. This mixture was kept in hot water bath for 10 minutes. The optical density was read at 610 µm against blank using UV-VIS spectrophotometer. Glycogen content is expressed in terms of mg glucose / 100 mg wet weight of tissue.

**Estimation of lipid (Barnes et al., 1973):**

100 mg tissue was homogenized by adding 10 ml of chloroform: methanol (2:1) mixture. The homogenate was filtered and one ml of this (filtrate) was kept at room temperature in laboratory at 37°C for 2 days to dry. 1ml of concentrated sulphuric acid was added to the dry mixture and kept in boiling water bath for exactly 10 minutes followed by cooling rapidly under tap water. 0.2 ml of this solution was taken and 5 ml Vanillin reagent was added and kept aside for 30 minutes at room temperature (37°C) before reading optical density at 530 µm using UV-VIS spectrophotometer. Lipid content expressed as mg lipid /100 mg wet tissue.

**Statistical analysis:**

The results were expressed as mean of three replicates and data were analysed statistically by using student ‘t’ test (Mungikar, 2003).
RESULTS

1. Protein

Total protein level in control earthworm Polypheretima elongata was found to be 68.2 mg% in skin; 45.7 mg% in ovary; 46.9 mg% in intestine, and 36.0 mg% in testis respectively.

In the sodium chloride (1.05g/kg soil) treated earthworm total protein level was found to be 42.7 mg% in the skin; 25.1 mg% in the ovary; 28.9 mg% in the intestine and 23.6 mg% in testis. Total protein level decreased significantly (P<0.05) by 37.39% in the skin; 45.8% in the ovary; 38.38% in the intestine and 34.44% in testis respectively (Table No. 3.1 Fig. 3.1).

2. Glycogen

Total glycogen level in control earthworm Polypheretima elongata was found to be 20.8 mg% in skin; 19.8 mg% in ovary; 21.6 mg% in intestine and 17.8 mg% in testis respectively.

In the sodium chloride (1.05g/kg soil) treated earthworm total glycogen level was found to be 18.8 mg% in the skin; 17.9 mg% in the ovary, 19.9 mg % in the intestine and 16.5 mg% in testis. Total glycogen level decreased significantly (P<0.05) by 9.62% in the skin, 9.6% in the ovary; 7.87% in the intestine and 7.3% in testis respectively (Table No. 3.2 Fig. 3.2).

3. Lipids

Total lipid level in control earthworm Polypheretima elongata was found to be 13.2 mg% in skin; 16.8 mg% in ovary; 20 mg% in intestine and 13 mg% in testis respectively.

In the sodium chloride (1.05g/kg soil) treated earthworm total lipid level was found to be 9.11 mg% in the skin; 12.1 mg% in the ovary; 15.2 mg % in the intestine and 8.7 mg% in testis. Total lipid level decreased significantly (P<0.05) by 33.07 % in the testis, 30.98% in the skin; 27.97% in the ovary and 24% in the intestine respectively (Table No. 3.3 Fig. 3.3).
DISCUSSION

Salinity change in the habitat of animal has been known to change behavioral and physiological activities. It is known that exposure of animals to salinity exerts physiologically dehydrated effect resulting in to various osmoregulatory changes. In other word salinity changes cause a lot of osmotic stress at the cellular levels of the animal (Sturzenbaum et al., 2005).

The present study is aimed to understand the sodium chloride induced alterations in the protein, glycogen and lipid metabolites in the earthworm *Polypheretima elongata*. This the earthworm when exposed to sublethal dose of sodium chloride (1.05g/kg soil) showed variation in the organic reserve of its tissues. It was observed that changes in these metabolites were tissue specific and reflected differential sensitivity of different tissues to sodium chloride toxicity.

Proteins are important group of macromolecular substances, which occupy a pivotal place in both structural and dynamic aspects of living matter (Harper et al., 1977). They are versatile macromolecules acting as biocatalyst, contractile tissues and form important constituents of body fluids.

The depletion in protein level was recorded in worms after 5 days of exposure to sublethal dose of sodium chloride in all the tissue. The maximum depletion was found in the skin followed by ovary, intestine and testis. Decrease in protein content may be due to degradation of proteins in to amino acids to be utilized for gluconeogenesis (Begum and Dharni, 1996) to mitigate the stress. Being a part of cell membrane and as an enzyme protein level might be decreased because of its metabolism to liberate energy during pesticide stress (Chaudhari et al., 1993).

It is established fact that proteins are used as an alternative source of energy especially under stress conditions. It may be suggested that in *Polypheretima elongata* also proteins must have been utilized for the production of energy to reduce the sodium chloride stress. These results are supported by several investigators who reported decline in protein content in different organisms under the influences of various stress (Li, 1992; Liu et al., 1995;
Glycogen serves as fuel and supply energy for metabolic process. Carbohydrates are used by cells in the form of glucose and utilize according to need of organism. The disturbances in the glycogen profiles are one of the most outstanding biochemical lesions due to the action of many chemicals (De Bruin, 1976). The possible mechanisms involved in the glycogen depletion are diverse in nature and such changes are frequently interpreted as specific general metabolic disturbances due to secondary stress conditions. The general glycogen depletion may also be due to activation of an enzyme glycogen phosphorylase, a major contributing factor to glycogen utilization. Glycogen, a reserve food and a reserve source of energy were found decreased during the entire exposure span in the earthworm, *Polypheretima elongata*.

The depletion in glycogen level in all the tissues of earthworm *Polypheretima elongata* could be due to general energy need of animal under stress condition. The maximum depletion was found in the intestine followed by skin, ovary and testis. Decrease in glycogen is attributed to its mobilization from muscle to coelomic fluid as a consequence of hypoxic or anoxic conditions. According to Reddy *et al.* (1984) and DeZwaan and Zandee (1972) carbohydrate metabolism gets altered during the stress and decrease might be due to the prevalence of hypoxic or anoxic conditions, which normally increase carbohydrate utilization (Hochachka, 1973; Reddy *et al.*, 1986).


Lipid is important dietary constituent, which serves as reserve energy when food supply is scanty. As the animal is starving fat depots are the
important source of energy. Lipid metabolism has important role under stress condition. After glycogen, lipids are used as stored energy source. The maximum depletion was found in the testis followed by skin, ovary and intestine. Decrease in lipid level suggests the mobilization of lipids for production of energy during stress caused by pesticides (Hochachca et al., 1973). It is a known fact that the decrease in lipid content correlates with increased activity of enzyme lipase, the enzyme responsible for the break down of lipids into free fatty acids and glycerol (Rajyalakshmi and Reddy, 1992).

The present results on earthworm *Polypheretima elongata* suggest that decrease in levels of protein; glycogen and lipid during exposure to sodium chloride may be due to increased glycogenolysis/glycolysis, proteolysis and lipolysis to meet energy demands.
Table No. 3.1: Protein levels in various tissues of *Polypheretima elongata* exposed to 24 h sublethal dose of sodium chloride for 5 days.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control</th>
<th>Experimental (1.05gNaCl/kg soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>68.2 ± 2.25</td>
<td>42.7 ± 1.21</td>
</tr>
<tr>
<td>Ovary</td>
<td>45.7 ± 2.1</td>
<td>25.1 ± 1.12</td>
</tr>
<tr>
<td>Intestine</td>
<td>46.9 ± 2.15</td>
<td>28.9 ± 1.14</td>
</tr>
<tr>
<td>Testis</td>
<td>36.0 ± 1.25</td>
<td>23.6 ± 1.19</td>
</tr>
</tbody>
</table>

Fig. 3.1: Percent change in protein levels in various tissues of *Polypheretima elongata* exposed to 24 h sublethal dose of sodium chloride for 5 days.
Table No. 3.2: Glycogen levels in various tissues of *Polypheretima elongata* exposed to 24 h sublethal dose of sodium chloride for 5 days.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control</th>
<th>Experimental (1.05gNaCl/kg soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>20.8 ± 2.21</td>
<td>18.8 ± 1.23</td>
</tr>
<tr>
<td>Ovary</td>
<td>19.8 ± 1.22</td>
<td>17.9 ± 1.15</td>
</tr>
<tr>
<td>Intestine</td>
<td>21.6 ± 1.18</td>
<td>19.9 ± 2.24</td>
</tr>
<tr>
<td>Testis</td>
<td>17.8 ± 1.15</td>
<td>16.5 ± 2.11</td>
</tr>
</tbody>
</table>

**Fig. 3.2:** Percent change in glycogen levels in various tissues of *Polypheretima elongata* exposed to sublethal dose of sodium chloride for 5 days.
Table No. 3.3: Lipid levels in various tissues of *Polypheretima elongata* exposed to 24 h sublethal dose of sodium chloride for 5 days.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control</th>
<th>Experimental (1.05gNacl/kg soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>13.2 ± 2.26</td>
<td>9.11 ± 1.14</td>
</tr>
<tr>
<td>Ovary</td>
<td>16.8 ± 2.12</td>
<td>12.1 ± 2.1</td>
</tr>
<tr>
<td>Intestine</td>
<td>20 ± 1.13</td>
<td>15.2 ± 1.4</td>
</tr>
<tr>
<td>Testis</td>
<td>13 ± 0.1</td>
<td>8.7 ± 1.17</td>
</tr>
</tbody>
</table>

Fig. 3.3: Percent change in lipid levels in various tissues of *Polypheretima elongata* exposed to 24 h sublethal dose of sodium chloride for 5 days.
REFERENCES


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