CHAPTER III MACROMOLECULAR AND RESPIRATORY CHANGES IN
DIAPAUSED EARTHWORMS

3.1. Introduction

3.2. Materials and Methods
   3.2.1. Estimation of macromolecules
   3.2.2. Estimation of total lipids and glycogen
   3.2.3. Homogenate respiration

3.3. Results
   3.3.1. Changes in DNA, RNA and protein contents
   3.3.2. Changes in lipid and glycogen contents
   3.3.3. Changes in homogenate respiration

3.4. Discussion
3.1. INTRODUCTION

Although biochemical composition and metabolic rate of animals are characteristics of the species, to a large extent these parameters depend on the physiological and/or developmental stages. These characteristics have considerable fluctuation in animals with life cycles interrupted by a period of dormancy, as a part of the development or as an adaptation to the environmental extremes. In diapause of insects like *Lucilia serricata* the protein to dry body weight ratio decreases significantly from the normal (Ring 1973). Earlier reports have shown similar changes in molluscs under stress conditions like anaerobiosis and starvation (Emerson 1967, Newell 1966). In estivating African lung fish *Protopterus*, changes in the metabolic rate (Smith 1930) and decline in protein content (Janssens 1964) are observed. A large number of reports are available in physiological and biochemical changes during estivation of gastropods (Meenakshi 1959, 1964, Reddy and Swamy 1976, Muralimohan and Sasirababu 1976 c, Krishnamurthy and Brahmpandam, 1970). In general, the metabolic rate in term of oxygen consumption decreases considerably during estivation or diapause. The nutrient source is normally limited to the breakdown products of stored molecules such as glycogen and lipids (Horne 1979).

As regards the body composition of the earthworm, the dry weight is about 15 to 20 % of the body fresh weight (Laverack 1963). The contents of macromolecules such as proteins, lipids, polysaccharides and nucleic acids have been estimated in many species of earthworms (French et al 1957, Dash et al 1977, Mishra 1982). In all the species proteins are the largest fraction of the dry weight, ranging from 73.76 % in Lampito mauritii (Dash et al 1977) to 53.5 % in Lumbricus terrestris (French et al 1957). Storage molecules like glycogen and lipids have been investigated in earthworms under various stress conditions. But no systematic analysis has been made to investigate the biochemical changes in earthworms during diapause. I have investigated the time course of changes in different body constituents and the metabolic rate, during diapause in Octochaetona surensis.

3.2. MATERIALS AND METHODS

Procedures for collection, rearing and induction of diapause in earthworms were as described in section 2.2.

3.2.1. Estimation of macromolecules

Tissues from three to five earthworms were homogenized in 10 ml of 10 % ice cold TCA by Potter Elvehjem homogenizer having glass mortar and teflon pestle. The homogenate was centrifuged at 6000 x g for 15 minutes in order to precipitate the macromolecules. Further isolations of different macromolecules were performed according to the scheme given in Fig.6.
Tissue homogenized with ice cold 10 % TCA (1:10) and centrifuged at 6000 x g for 10 min

Residue: washed with five volumes of 5 % TCA by centrifuging at 6000 x g for 5 min

Supernatant: added five volumes of 10 % TCA and centrifuged at 6000 x g for 10 min

Residue: Residue

Supernatant: discarded

Washed with ethanol, by centrifuging at 6000 x g for 10 min

Residue: washed with ethanol-chloroform (1:2) by centrifuging at 6000 x g for 10 min

Supernatant: discarded

Residue: washed with diethyl ether by centrifuging at 6000 x g for 10 min

Supernatant: discarded

Residue (contained RNA, Protein and DNA): airdried, added 0.1 N NaOH (1:2) and shaked continuously for 3.5 h in a water bath at 37°C. To it added 20 % TCA and centrifuged at 6000 x g for 10 min

Supernatant: discarded

Residue: heated with 20 % TCA at 90°C for 20 min, cooled and centrifuged

Supernatant: estimated for RNA

Residue: dissolved in 1 N NaOH, after little warming and estimated for protein

Supernatant: estimated for DNA

Fig. 6 Flow sheet for macromolecular isolation.
The amount of DNA was measured by the method of Dische (1930) using diphenylamine reagent containing diphenylamine, glacial acetic acid and conc. \( \text{H}_2\text{SO}_4 \). The DNA solution was heated at 90°C for 15 minutes with diphenylamine reagent (1 : 3) and after cooling was read at 600 nm by a spectrophotometer (Spectronic 20, Bousch and Lomb U.S.A.). The amount of DNA was determined by a standard DNA solution.

The estimation of RNA was done by the orcinol reagent containing \( \text{FeCl}_3 \), conc. \( \text{HCl} \) and 1 % ethanolic orcinol as described by Schimdt and Thannhauser (1945). Color development was done by heating the RNA solution with orcinol reagent (1 : 2) for 20 minutes at 100°C and the optical density was measured at 660 nm. The RNA content was determined from the standard graph.

Protein content was measured according to the principle of Folin-Ciocalteu reaction (Lowry et al 1951). The protein solution was mixed with protein reagent (1 : 5) containing \( \text{Na}_2\text{CO}_3 \), \( \text{CuSO}_4 \), Na-K tartarate and \( \text{NaOH} \), and thoroughly shaken by a cyclomixer. After 40 minutes, 0.5 ml of Folin's reagent (having sodium molybdate, sodium tungstate, phosphoric acid, conc. \( \text{HCl} \) and lithium sulphate) was added to it and incubated for 15 minutes. The color complex was measured at 750 nm and the protein content was determined by the help of a standard graph.

3.2.2. Estimation of total lipids and glycogen

Five earthworms were taken for lipids and glycogen estimation. Total lipid content was determined by gravimetric method after extraction with chloroform-methanol, 2:1 (Folch et al 1957). Glycogen was estimated by the method of Hasid and
Abraham (1957). After isolating it in 95% ethanol the amount of glycogen was measured by anthrone reagent (0.2% anthrone in conc. H₂SO₄) method and the optical density was measured at 660 nm.

3.2.3. Homogenate respiration

Tissue homogenates of individual earthworms were prepared in earthworm ringer (Wilson 1979) and oxygen consumption was measured by the method of Kale and Rao (1973) at 25°C using oxygen analyser (Universal Biochemicals, Madurai, India) fitted with Clark type electrode.

3.3. RESULTS

3.3.1. Changes in DNA, RNA and Protein content

Changes in DNA content on different days of diapause is given in Fig. 7. It was observed that the amount of DNA did not vary significantly during diapause.

Fig. 8 shows the diapause induced changes in RNA content of O. surensis. The loss in RNA content was 29% of the initial value in 30 days. The loss in first two weeks was significantly higher than that in the subsequent period. The RNA to DNA ratio also depicts a similar trend (Fig. 8 inset).

Changes in protein content during diapause is shown in Fig. 9. The loss in protein content was similar to the loss in RNA. During first 15 days of diapause, 35% of the initial content was depleted as compared to 7% loss in the later 15 days. However, loss in protein was more than the RNA during diapause. This is
Fig. 7 Changes in the DNA content during diapause in earthworm. The initial value of 100% on zero day is equal to 3.93% dry weight of the worm.
Fig. 7

Days of diapause vs. % initial content for the study.
Fig.8 Changes in the RNA content during diapause in earthworm. The initial value of 100% on zero day is equal to 19.75% dry weight. The inset of figure shows the changes in the RNA to DNA ratio.
Fig. 8
Fig. 9 Changes in the protein content during diapause in earthworm. The initial value of 100% on zero day is equal to 43.83% dry weight of the worm. The inset of the figure depicts the changes in protein to RNA ratio.
Fig. 9

% initial content

days of diapause

protein/RNA

2.5

2.0

1.5

15

30
also evident from the protein to RNA ratio as shown in Fig.9 inset.

3.3.2. Changes in lipid and glycogen contents

A significant decrease in both lipid and glycogen contents was observed in diapausing *Q. surensis* (Fig.10). The amounts of lipid lost by 15th and 30th day diapause were about 36 and 48% of the initial respectively. On the other hand, 29% of the initial glycogen content was lost by 15th day and 47.3% by 30th day of diapause. It can be further observed that the glycogen depletion was more than the lipids in the later part of dormancy.

3.3.3. Changes in homogenate respiration

Table 8 depicts the changes in oxygen consumption of the tissue homogenate during diapause. It decreased from an initial value of about 186 mm$^3$ of O$_2$ per g fresh weight per hr to 52 mm$^3$ and 39 mm$^3$ of O$_2$ on 15 and 30 days of diapause respectively. When converted to energy equivalent (according to Engelman 1961) the decrease is from $883 \times 10^{-6}$ kcal per h to $187 \times 10^{-6}$ Kcal per h by the end of 30 days of diapause.

3.4. DISCUSSION

Macromolecular estimations in *Q. surensis* during diapause reveal that excepting DNA, all other types of macromolecules decline considerably from the normal levels. The relative stability of DNA suggests that there is no cell death or degeneration during diapause. On the other hand, a decline in contents of macromolecules such as protein and RNA indicate inefficiency of the
Fig. 10  Changes in the total lipid and glycogen content during diapause in earthworm. The initial value of 100% on zero day is equal to 13.43% and 9.67% of dry weight respectively for total lipid and glycogen.
Table 8. Oxygen consumption by tissue homogenate of diapausing *O. surensis*.

<table>
<thead>
<tr>
<th>Days of diapause</th>
<th>( O_2 ) consumption in mm(^3)/g fr wt/h</th>
<th>(^+)Energy equivalent ( \times 10^{-6} ) kcal/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>167 - 205</td>
<td>802 - 984</td>
</tr>
<tr>
<td>15</td>
<td>42 - 61</td>
<td>202 - 293</td>
</tr>
<tr>
<td>30</td>
<td>29 - 49</td>
<td>139 - 235</td>
</tr>
</tbody>
</table>

\(^+\)Energy equivalent is calculated as 4.8 kcal/liter of oxygen (Engelmann, 1961).
diapausing animal to maintain the levels of essential macromolecules. However, it is not possible to establish the precise mechanism of these changes due to lack of detailed experimental evidences.

The storage molecules namely glycogen and lipids show a steady decline during diapause. The rate of depletion of glycogen is faster than the lipids during the later part of diapause. In many invertebrates glycogen is the major nutrient source during anaerobiosis and starvation (Emerson 1967, Zwan and Zandu, 1972). In earthworms also glycogen is the storage carbohydrate and is the chief source of nutrition during starvation (Laverack 1963, Dales 1969). It normally acts as an energy pool stored in chloragogen cells, from which it is mobilised to meet the nutritional requirements of starved earthworm (Van Gansen 1956). In Tubifex a large quantity of glycogen is depleted during the period of anaerobiosis (Dausend 1931). Waraska et al (1980) reported that in Lumbricus terrestris glycogen reserves decline from an average of 14 mg/g fresh weight to 4 mg/g wet weight during starvation.

Lipids in earthworms as in other systems might have manyfold functions. Besides being the important components in biomembranes they are also present as functional and storage lipids. The later role of lipids is evident in some polychaetes, where several fatty acids were found accumulated in the tissue under anaerobic conditions. But, the function of these fatty acids as precursor for energy production is limited due to their oxygen dependent degradation inside the mitochondria. A metabolic pathway which demands relatively large amount of oxygen may not be preferred
under such metabolic condition. Moreover, during the period of diapause the worm experiences near anaerobic condition being encapsulated by a cell. In these circumstances energy requirements for its maintenance can only be met by anaerobic breakdown of glycogen. Therefore it may be plausible that in diapausing earthworm, major source of energy is glycogen, whereas the role of other compounds like lipids may be secondary. The fatty acids, degradative product of complex lipids, might be involved in regulation of energy yielding functions like oxidative phosphorylation.

A decrease in metabolic rate is the characteristic of the state of dormancy in animals. Earlier reports reveal drastic reduction in oxygen consumption in estivating animals (Smith 1930, Meenakshi 1959). In the present investigation significant (about 80%) decrease in the oxygen uptake rate by tissue homogenates of O. surensis is observed in a period of 30 days. The decline in metabolic rate has a strategic significance for the maintenance of the dormancy in earthworms. The adaptiveness of the change is evident from the fact that the conservation of the nutrients, derived from the degradation of body constituents can be achieved by their decreased utilization in respiration. Alternatively, the limited amount of oxygen in the ambient environment (cell) might influence the oxygen uptake rate of the earthworm. In either of the possibilities, a decrease in metabolic rate has a distinct survival value for the worm during its prolonged inactive period.