CHAPTER V  GENERAL DISCUSSION

5.1. Characteristics of induced diapause in earthworms

5.2. Strategy for survival during diapause

5.3. Strategy for reversal of diapause

5.4. Nature of diapause at molecular level
5.1. Characteristics of induced diapause in earthworms

Simulating conditions for laboratory induction of diapause in earthworms have been standardized for the first time in the present investigation. Similarities between the induced and natural diapause are evident from the morphological and structural changes. Morphological changes like formation of a cell, coiled and inactive body (Fig. 1) are identical to the naturally diapaused worms (Michon 1954). Loss in body weight is another characteristic similar to that in natural diapause. The worms lose 60% of their body weight (Fig. 5, Table 7) during 30 days of diapause, which includes 47% loss in body water and 62% loss in other body constituents over the normal. Due to the loss in dry weight there is apparent stability in percentage moisture of diapausing worms.

The loss in dry weight can be correlated with the macromolecular depletion during diapause. Decreases in the content of macromolecules like RNA, protein, glycogen and lipids contribute to the dry weight loss of the worm (Figs. 7-10). The changes are similar to that in other dormant animals (Janssens 1964, De Kort 1969, Ring 1973). The loss in these molecules might be related to the supply of nutrients to the starving worm. Since there is no external supply of nutrients, the degradative products of the macromolecules might be utilized for energy production. The changes in the content of these molecules during 30 days diapause show differential rates in first and second 15 days. In the later half, the loss in RNA content is insignificant and the loss in other molecules are significantly
lower than the earlier 15 days period. A similar trend in loss of dry weight is suggestive of its relationship with macromolecular loss during diapause.

Another characteristic of the induced diapause similar to that in natural conditions, is the significant reduction in the rate of oxygen consumption (Table 8). The low rate of oxygen uptake shows the adaptation of the worm for energy conservation during diapause. Earlier reports reveal similar features in other diapausing animals (Meenakshi 1964, Stegwee 1964). The respiratory loss during diapause have also been evident from the changes in mitochondrial structure and function (Figs. 23-26, Table 12-14). Studies on the changes in mitochondrial structure reveal diapause induced changes in heat sensitivity and alterations in the permeability properties of the membrane. The changes in membrane configuration tend to reduce the functional efficiency of mitochondria. A significant reduction in the ADP/O ratio and complete loss in respiratory control after 15 days, reveal a loss in phosphorylating ability of mitochondria. These findings corroborate with the earlier reports on mitochondrial changes in diapausing insects (Stegwee 1964, De Kort and Bartelink 1972). The damage to the mitochondrial membranes during diapause is also evident by the loss in electron transport ability indicated by a decrease in specific activity of succinate dehydrogenase. Similar changes have been reported in diapausing molluscs and insects (Eckstein and Abraham 1959, De Kort 1969).
The changes during earthworm diapause reveal a pattern of adaptation in which the changes during the initial 15 days are significantly different from the later period. During the initial period, when the respiratory rate has not been stabilised at a lower level, the rate of macromolecular loss remains relatively higher (Figs. 8,9,10). In later stage the worm adapts to a low rate of respiration possibly by switching over to anaerobic mode of energy production. It conserves its nutrients by decreasing the rate of macromolecular depletion to a lower level.

5.2. Strategy for survival during diapause

The general adaptational strategy of the diapausing earthworm can be reconstituted from observations in the present investigation. These results depict an intrinsic pattern, which can be correlated with the survival mechanisms of the earthworm. An essential feature of adaptation during diapause is the formation of a cell or capsule. The cell being closed and air tight might function as a protective shield around the coiled worm and might maintain a higher relative humidity than its surrounding, preventing undue loss in body moisture. At the same time it might function as an envelop limiting the oxygen content in the ambient environment of the worm. As a result, the worm adapts to low pO₂ and reduces the metabolic rate drastically. The long term adaptation of the worm results in a significant decline in the aerobic respiration. Under these circumstances the energy for its survival is mainly derived from the degradation of storage molecules namely glycogen and lipids. But
the relatively slow rate of depletion of these molecules during the later 15 days of diapause, indicate the strategy of the worm for survival. By decreasing the rate of loss it can conserve the contents of storage molecules for a longer period.

Mitochondrial changes during diapause also reveal adaptation at cellular level for the survival of the worm. The low level of mitochondrial respiration, loss in phosphorylation and electron transport constitute the adaptational strategy for reduction of respiratory rate. Alterations in the permeability properties of mitochondrial membranes also reflect a possible mechanism by which it might restrict the entry of active metabolites into it and might be able to maintain a lower rate of oxygen uptake.

Another important feature in the diapausing earthworm is the loss in body water (Table 7). A loss in metabolic water puts strain on the enzymatic pathways due to the alteration in enzyme-substrate affinity in an environment of low solvent concentration. In diapausing worm the body water loss might be an adaptation to decrease the metabolic rate to a level at which it can survive for a longer period in a limited substrate content.

5.3. Strategy for reversal of diapause

Diapause in animals has a survival value because it terminates with the reversal to active life on the return of favourable conditions. In some animals like the earthworm *O. surensis* it is almost immediate or without a lag period. For the quick reversal, the adaptational features must reflect a
degree of stability in some essential components, which can function immediately after the termination of diapause. Several features of diapause in _O. surensis_ reflect the reversal strategy of the worm.

The most important observation in this regard is the stability in DNA content of the worm during the period of diapause. It may suggest that during diapause the number of cells remain constant and are capable of growth and reproduction on the termination of diapause. Another observation which depicts the reversal strategy is the relative stability of RNA content compared to protein, after 15 days of diapause. These stable RNA molecules might be essential for the recovery process and might function instantaneously on the return of favourable conditions. Possibly a fraction of the RNA might be present in the form of polyribosome-mRNA complex which are relatively immune to the action of degradative enzymes. Earlier studies in seed dormancy reveal that the mRNA for synthesis of some essential enzymes during germination are preformed and stored (Walbut et al 1974, Walbut 1978). During germination these mRNA are polyadenylated for immediate translation to synthesize essential enzymes.

In the alterations of mitochondrial functions the survival strategy may also be observed. Changes in ADP/O and 'respiratory control index' do not necessarily reflect permanent damage of the mitochondrial membrane. Earlier reports have shown that mild treatments with chemicals like insulin may cause loss of respiratory control, but can be restored by addition of hormones such as thyroxin (Katkocin et al 1979, Clejan et al 1980).
5.4. Nature of diapause at molecular level

In earthworms, the phenomenon of diapause can be investigated at behavioural, morphological, physiological and molecular levels. But its analysis at the molecular level provides deeper understanding of the adaptational process of the animal. The studies in mitochondrial membranes might be used as a model reflecting general pattern of adaptation at membrane level. The permeability changes and functional alterations in the mitochondrial membranes might reflect adaptations at the microstructural level of the cell. The changes in permeability properties of the membrane might selectively control some specific metabolic processes regulating the organelle during diapause. Similarly, studies in membrane function such as oxidative phosphorylation provide information regarding the reversibility of diapause.

Eventhough there are several differences in the composition and ultrastructure of biomembranes, the basic plan of the entire membrane system remains the same. Thus, in the present investigation, the studies in mitochondrial membrane may be regarded as a model system for molecular analysis of diapause. Elaborate studies of mitochondrial and other membrane systems during diapause merit further investigation.