CHAPTER V
DISCUSSION
In the age of industrialization, man is exposed to many new chemicals or toxins in a more prevalent manner. Physiology of an animal depends greatly on their metabolic functions. In this respect the present investigation reveals a positive correlation to aerobic and anaerobic status with respect to the endosulphan accumulation in the experimental context in a mammalian representative, albino mice.

The effect of acute, subchronic and chronic exposure of Endosulphan at the rate of 6 mg / kg body weight once daily for 21, 60 and 90 days on female albino mice were investigated. The behavioural changes or clinical signs and biochemical variations in the various metabolites like glycogen, protein, lipid and the enzymatic variations, histopathological changes, variations occurring to endosulphan which is converted into isomers like alpha Endosulphan, beta endosulphan and endosulphan sulfate in brain, liver and kidney of female albino mice Mus musculus, have been evaluated.

In the present study, the body weight at every day was taken to find out the effect of insecticide on growth rate, glycogen, total protein and total lipid were assayed to identify the effect of endosulphan on carbohydrate, protein and fat metabolism. Metabolism is a complex network of chemical reactions occurring in several different compartments in eukaryotic cells. It involves a bewildering array of chemical reaction. Many of them organized as complex cycles which may appear difficult to understand (David, 1994; Jens and Braunbeck, 2001).

Besides these major energy reserves, a set of enzyme activities such as alkaline phosphatase and acid phosphatase (as a marker for oxiradical
scavenging) adenosine triphosphatase (chief enzyme involved in energy metabolism), lactic dehydrogenase and succinic dehydrogenase (as markers of citric acid cycle and anaerobic respiration) and acetylcholinesterase (a specific marker of organo chlorine toxicity) were studied.

Alterations in metabolic enzyme activities were able to serve as a tool for the sensitive identification of environmental pollutants which in turn form the basis for an improved understanding of underlying toxic processes and an interpretation of the toxic related effect (Konradt and Braunbeck, 2001). Acid and alkaline phosphatases were estimated to see the cellular damage and tissue irritations in brain, liver and kidney due to the exposure of endosulphan. Total ATPase, succinic dehydrogenase and lactic dehydrogenase were estimated to see the effect of endosulphan on energy metabolism. Acetylcholinesterase activity were, measured to understand the excitatory activity of endosulphan or check the effect of endosulphan in the control and co-ordination. Histopathological (brain, liver and kidney) parameters and residue analysis were studied to evaluate the extent of organ toxicity caused by endosulphan at the administered doses.

The LD$_{50}$ for endosulphan utilized for present study was calculated as 7.36 mg/kg body wt. (Finny, 1971; Worthing, 1987). The two isomers of endosulphan also have different LD$_{50}$ values in mice. The alpha isomer is more toxic than the beta isomer. In female albino mice, the lethal dose for alpha endosulphan being 11 mg/kg body wt. and that for beta endosulphan is 36 mg/kg/body wt. (Dorough et. al., 1978). The lethal dose for endosulphan sulfate in mice were comparable to that of the alpha isomer, 8 mg/kg body wt. (Dorough et. al., 1978) had reported an LD$_{50}$ of 14 mg/kg/body wt. for alpha
endosulphan and 17 mg/kg/body wt. for beta endosulphan in female mice. The same difference was reported in female rats, with an oral LD$_{50}$ value of 76 mg/kg/body wt. (for alpha isomer) verses an LD$_{50}$ value of 240 mg/kg/body wt. for beta endosulphan (Maier-Bode, 1968; Antonious et. al., 1998). Cole, et. al., (1994) found LD$_{50}$ for house flies of 1.34 µg/g for alpha isomer and 3.24 µg/g for beta isomer. In the present study, causality of the subject been noticed from 90 day onwards. Female mice are more sensitive to endosulphan than male.

A progressive increase in the body wt. of control and a decrease in the body wt. of treated group were observed. The slight increase in the body wt. of control may be due to the fact that the mice were in the growing age. From the second week of treatment until the termination of the experiment, treated mice lost weight comparatively, which is significant.

Decrease in body weight in endosulphan treated mice has been attributed to the impairment in food assimilation and the tissue damage resulting in decreased appetite and absorption of nutrients from the gut (Venkateshwarlu e.t al., 1997; Thaker and Garg, 1993; Siddiqui, 2004; Sing et. al., 1998; Jayasree et. al., 2003). The decrease in body weight might be an indication of direct toxicity or stressogenic activity of these compounds (Gowda et. al., 1983; 1984; Lakshmana and Raju, 1994). The reduction in weight also could be due to increased anaerobic pathway induced by endosulphan intoxication.

Histopathological study reveals the tissue inflammatory reactions and focal necrosis occurs in the tissue. This is due to the cumulative toxic effect.
exerted by endosulphan. This cumulative toxic effect exerted by endosulphan might decrease body weight and food intake in treated animals. (Nasser and Zanty, 1994; Jabeen, 1984; Quadri et. al., 1987)

According to Sinha et. al., (2000) and Gupta and Chandra (1997), rats treated with 10 mg endosulphan / kg/ day by gavage in oil for 15 days gained 30% less weight than control. Decreased body weight was reported in clams treated with doses of 3.8 mg / kg/ body wt./ day for 84 days. A dose of 2 mg technical endosulphan / kg/ day by gavage in water for 90 days was also reported to cause significant reduction of weight gain in rats (Paul et. al., 1994) along with this food intake was also suppressed. The present study also shows the same results.

5.1 Glycogen

A high carbohydrate diet usually leads to a decreased rate of detoxification. The microsomal oxidation is generally depressed when the carbohydrate/protein ratio is increased. In addition, the nature of carbohydrate also affects oxidase activity. Since dietary carbohydrate influence the body lipid composition, the relationship between carbohydrate nutrition and toxicity is often difficult to assess. However, the environmental chemicals can affect and be affected by body glucose metabolism (Devi, et. al., 1981). It is widely accepted fact that glycogen is an important macromolecule which comes first to the reserve for mice by providing energy from entowering stress caused by any xenobiotic (Lal, et. al., 1984; Dange, 1986).

The function of glycogen in animal cells is to store the metabolic fuel glucose and to release it rapidly when needed. Glucose must be stored as a
polymer, because glucose itself could not be stored without a drastic increase in intracellular osmotic pressure (Browner and Fletterick, 1992). It has been estimated that total concentration of glucose residues stored as glycogen in a liver cell is ~ 0.4 M, whereas the concentration of glycogen is only ~ 10 nM. This huge difference mitigates osmotic stress (Cori, et. al., 1939). To fulfil its biological functions, the glycogen polymer must store largest amount of glucose in the smallest possible volume while maximizing both the amount of glucose available for release by glycogen phosphorylase and the number of non-reducing ends (Hevia, et. al., 1993).

The glycogen metabolism utilizes anaerobic glycolysis and produces a greater fraction of total ATP utilized (Rao, 1973). Glycogen the anaerobic fuel, the readily available source of energy during stress conditions. Usually most vertebrates are aerobic organisms which first convert glucose into pyruvate by glycolysis and oxidize pyruvate completely to CO₂ and H₂O using molecular oxygen (Voet, et. al., 2006).

In the present observations of the glycogen estimation in the brain, liver and kidney of the experimental group shows significant reduction when compared to control group, since, stress imposes an increased energy requirement from the animal. This is achieved through breakdown of reserve store of glycogen, in order to meet high energy demand of such stress. Similar decrease in glycogen content on exposure to pesticide have been reported by Bakthavalsalam and Reddy (1993) in Anabas testudiensis, Rao (1989) in Catla calta and Gill and Pand (1991) in Barbus conchonius. Similar phenomenon was observed in eels exposed to Lindane (Ferrando and Andreu, 1992). Singh and Srivastava (1981) in a series of experiments observed significant and
persistent hyperglycemia in endosulphan exposed fish *Heteropneustes fossilis*. Different studies have shown that the stress produced by acute pesticide exposure (Holmberg, *et. al.*, 1972; Hanke, *et. al.*, 1983; Ferrando, *et. al.*, 1989 a,b) is accompanied by rapid depletion of liver and muscle glycogen reserves and a significant elevation of blood glucose.

Breakdown of glycogen pool also supplies the increasing energy requirements for detoxification processes as well as for the synthesis of conjugates like glucuronic acid which further help in the elimination of pesticide and their metabolites (Singh, *et. al.*, (1998). Stressful stimuli elicit rapid secretion of both glucocorticoids and catecholamines from the adrenal tissue of organisms; both hormones produce rapid hyperglycemia (Singh and Srivasthava, 1981; Dange and Masurekar, 1982). The liver glycogenolysis observed in endosulphan exposed eels suggests a typical stress response confirming the prevalence of a hypoxic condition at tissue level since anoxia or hypoxia increases carbohydrate consumption (Van Waarde, *et. al.*, 1983; Thillart and Smith, 1984). Decrease in glycogen content in various tissues of *Oreochromis mossambicus* was observed (Abhilash and Prakasam, 2006).

Depletion of glycogen may be due to direct utilization of this component for energy generation. Glycogen rapidly catabolized resulting in a rapid decrease in this energy reserve (Sancho, *et. al.*, 1998; Rambabu and Rao, 1994). Thus, in the present study, the decrease in glycogen content in various tissues of *Mus musculus* might be thus related to the metabolic adaptation of the mice to its changed internal environment or homeostasis. Poisoning by chemicals may damage the membrane environment of the enzyme during detoxification. Compounds that are metabolized to glucuronyl conjugates are
more toxic to the individuals especially the components of the anti oxidant
defence system (Waneene, et. al., 2002).

5.2 Total Protein

Proteins are naturally occurring polymer of high molecular weight
consisting predominantly of amino acids linked by peptide bonds. Proteins
account for more than 50% of the organic constituent of protoplasm. They are
the major component of the dry material by weight of the living organisms and
they are among the most important functional components of the living cell.

In our study the observations indicate the disruption of protein
metabolism in tissues such as brain, liver and kidney of mice exposed to sub
lethal concentration of endosulphan. It would be seen that pesticide toxicity
stimulate proteolysis in tissues by activating protease enzymes. Protein
depletion in tissues may constitute a physiological mechanism and may play a
role of compensatory mechanism under pesticidal stress to provide
intermediates to kreb cycle or to enhance osmolarity of the body fluids during
the pesticidal stress (Yasmeen, 1986; Rajeswari, 1986).

Structural proteins contribute to mechanical structure of organs and
tissues or they may constitute the bulk of a natural structure. Contractile
proteins are responsible for active movements of living organisms. Proteins
are also involved in biochemical defenses (Antibodies, interferons etc) Rao
and Ramaneswari, 2000). The decrease in protein content of organochlorine
intoxicated ispods also indicates a physiological adaptability to compensate
for pesticide stress (Riberio, et. al., 2001).
The present observations corroborated with many previous studies. The decline in problem may be related to impaired food intake, increased energy cost of homeostasis or detoxification during stress. The detoxification process of endosulphan may lead to disruption of active site of proteins and structural variations in the aminoacids or proteins by binding up of the complex molecules. To overcome the stress condition, animal require high energy and this energy demand may have led to the utilization of these energy yielding compounds, that is, gluconeogenesis increases to meet the high energy demands. Decrease in protein content may also be due to a mechanism of lipoprotein formation which will be used to repair damaged cells and tissue organells (Sancho, et. al., 1998; Rambabu and Rao, 1994; Van Brummelen and Stuijifxand, 1993; Van Brummelen, et. al., 1996, a; Reddy, et. al., 1991). According to Praveen, et. al., (1987) it is probable that the protein is catabolized for entry into TCA cycle to cope with energy demands augmented during pesticide stress. Various proteins are involved in blood clotting system (Kozutsumi, et. al., 1998). The effect of proteins on pollutant toxicity includes both quantitative and qualitative aspects. Experiments show that animals fed proteins of low biological value exhibited a lowered microsomal oxidase activity (Riberro, et. al., 2001).

In particular many organochlorine pesticides have been known to suppress the protein concentration in tissues. The same effect was reported in various animal models and fish like Cirrhinus mrigala (Murthy and Devi, 1982) Channa punctatus (Swaroop, et. al., 1981) exposed to endosulphan. Short-term exposure of Oziotelphusa senex senex to endosulphan caused a significant reduction in total proteins of gills and various tissues of fish
(Naidu, 1985). Depletion of tissue proteins in animal models exposed to various toxicants have been reported by several investigators (Ramlingam and Ramlingam, 1982; Rao and Rameswari 2000). The decrease in protein content of organochlorine intoxicated isopods also indicates a physiological adaptability to compensate for pesticide stress (Riberio, et. al., 2001)

5.3 Lipid

Lipids may affect the toxicity of the environmental chemicals by delaying or enhancing their absorption. The endoplasmic reticulum contains high amounts of lipids, especially, phospholipids, rich in polysaturated fatty acids. Lipids may influence the detoxification process by affecting the cytochrome P-450 system because phosphatidyl choline is an essential component of the hepatic microsomal MFO system (Mosmann, 1983).

Dietary lipids play a unique role in the toxicity of chlorinated hydrocarbon pesticides. Dietary lipids may favour more absorption of these pesticides, but once these chemicals are absorbed into the body they may be stored in the adipose tissue, without manifestation of toxicity (Murthy and Devi, 1982).

In the present study, the results of lipid changes in various tissues of mice on exposure to endosulphan showed that all the exposed tissue registered a general decline in lipid content in response to increase in duration. The changes in the lipid content of the tissue might be attributed to the reduced rate of lipid synthesis as suggested by Saxeena, et. al., (1989). Decrease in lipid content might also be due to gluconeogenesis, over the stress condition in animal due to endosulphan toxicity. Endosulphan may cause disruption in lipase

A significant decrease in lipid and glycogen content were observed in P. dilatatus (Riberio, et. al., 2001; Sancho, et. al., 1989 and Rambabu and Rao, 1994). Total reduction in lipid due to endosulphan toxicity in major carp Catla catla especially in liver, brain and kidney were reported. The present observation are in line with the previous studies.

During the metabolic adaptation of the animal a purposeful rearrangement of physiological and biochemical functions takes place which help the animal to survive and also to adapt to extreme environmental conditions. Thus, in order to meet the basic energy requirements the organisms under the pesticide stress probably might increase their metabolic rate resulting in the accelerated utilization of tissue energy reserves as concluded from the present study.

5.4 Alkaline and Acid Phosphatase (ALK and ACP)

Phosphatases of diagnostic significance are of two kinds alkaline phosphatase (ALP) and acid phosphatase (ACP). These are differentiated by their reaction in the alkaline and acid medium. The alkaline phosphatase and acid phosphatase are non-specific and cleave many different phosphatase
esters. The non specific phosphatase may provide inorganic phosphate ions in place where they are needed. All phosphatases help to derive metabolic cycles.

Phosphatases are enzymes that are widely distributed in nature and characterized by their ability to catalyze the hydrolysis of phosphoric acid esters (Muller, 1981; Winn, 1988).

Present study shows duration related changes of ACP and ALP. The concentration of ALP and ACP were found to be higher in all exposed tissues. Pollutant stress is shown to increase the activities of various enzymes such as proteases, lipases, acid phosphatases and alkaline phosphatases in a body tissues of animals (Laws, 1981). The phosphatases play an important role in acute energy crisis in many organisms and involve in cytolysis and differentiation processes. In-order to overcome the stress conditions, these enzymes increases its activity in the cells (Nagarajan and Suresh, 2005; Siddiqui, et. al., 1987).

The increase in ACP and ALP revealed that energy metabolism is enhanced by substrate phosphorylation. This may be the reason for the increase in ACP and ALP. Along with this, inflammatory and necrotic changes at the acute exposure were also noticed. Granulation or pigmentation at subchronic exposure and degenerative changes were noticed in chronic stage. Depletion of energy reserves like glycogen, lipid and total protein may induce the increased production of ACP and ALP to overcome the depletion of energy. It may be due to the inflammatory and necrotic changes induced by endosulphan.
Endosulphan was reported to cause inflammatory and necrotic changes at cellular level (Bhavan and Geraldine 2000) resulting in the rupture of cellular and lysosomal membranes and in effusion of their contents, ultimately leading to increased levels of phosphatases. Increased levels of phosphatase have been noticed in the fish *Puntius conchonius* after exposure to endosulphan (Gill, *et. al.*, 1990), in prawn *Macrobrachium lamarrei* (Omkar and Shukla, 1985) and in the Shrimp *Callianassa tyrrhena* (Thakker and Haritos, 1989).

Alkaline phosphatase is an ectoenzyme and is associated with transport mechanisms in liver, kidney, intestine and blood. In animals ALP are found in the brush bordered epithelium of kidney cells, intestinal mucosa, osteocytes and osteoblasts of bone (Fishman and Ghosh, 1967). In the present study the increased alkaline phosphatase activity were noticed in liver, brain and kidney of *Mus musculus*. Moderate increase in alkaline phosphatase level is seen in hepatotoxicity and renal toxicity (Chaudhari, 2005).

The alkaline phosphatase requires Zn\(^{++}\) which is allosterically activated by Mg\(^{++}\) and has p\(^{H}\) optimum (Craig, *et. al.*, 1996). The active site contains two Zn\(^{++}\) ions and one Mg\(^{++}\) ion which are held by imidazole and carboxylate group. The inorganic phosphate is an enzyme product complex bound to Zn ions. Thus it is usually regarded as the metalloenzyme (Fishmann and Ghosh, 1967) and suggested that the -NH\(_2\) group –SH and a metal group are essential for the action of catalytic reaction (Linn, *et. al.*, 1976). Changes in the alkaline phosphatase has been associated with chemical treatment which create hepato cellular toxicity as Zn\(^{++}\) is important component and it is essential for enzymatic activity. The removal of Zn\(^{++}\) by chelators cause inhibition or loss
of enzymatic activity. Thus, influence of Zn$^{++}$ is supposed to provide a proper conformational change (Schleisinger, 1966) which may lead to variation in enzyme activity.

Acid phosphatase is a lysosomal enzyme (de Duve, 1959) and also found in pigment bodies, golgi apparatus, glial cells and in the endothelial cells (Kreutzberg and Hagger, 1966). Acid phosphatase is also considered as an enzyme of general cell metabolism (Manocha, 1970).

As ACP is a lysosomal enzyme, the important role of lysosomal enzymes as determined from the study of other tissues appeared to be hydrolysis and tissue repair (Unakar, et. al., 1973, 1975, 1978, 1982 and 1985). Hydrolytic enzyme have been postulated to play an essential role in the break down of extra cellular material under pathological conditions and tissue repair (Dingle, 1969). These enzymes involve in the tissue re-organization that accompanies the tissue repair in the pathological conditions, and an increase in the acid phosphatase activity was reported by Gorthy (1978). These enzymes are involved in the cell membrane degradation in toxic conditions (Gorthy, et. al., 1978).

From the present study it can be observed that the concentration of ACP increased depend upon the dose and duration. Similar results and observations were reported in prawn *Macrobrachium malcomsonii* on exposure to endosulphan (Bhavan and Geraldine, 2000) in fish *Puntius conchonius* (Gill, et. al., 1990) in prawn *Macrobrachium lamarrei* exposed to Dichlorvos (Omkar and Shukla, 1985).
Acid phosphatase plays a vital role in the autolytic degradation of dead cells. In the present study the elevation noted in ACP level correlated with cell necrosis and suggests that this was necessary to participate in dissociation of severely damaged cell in the tissues exposed to endosulphan. It is clear that an increase was observed in ACP activity during pathogenesis probably to remove the injured tissues and to enhance the repair mechanism. The normal epithelial cells synthesize lysosomal enzymes and synthesis of ACP is accelerated during the injury of tissues (Unakar, et. al., 1985). Histological and residue analysis supports these facts. Unlike other tissues in which polymorphonuclear leucocytes have been shown to infiltrate the damaged tissue, these cells become a source of acid hydrolases. (Unakar, et. al., 1973, 1975, 1985). A significant increase in ACP activity revealed that as damage increases more ACP is synthesized by the cells as an effort to remove the tissue debris and to prevent further damage. It is possible that acid hydrolases play a role in the process of tissue remodelling and reorganization that accompanies ongoing proliferative and degenerative changes during pathogenesis.

5.5 Total Adenosine Triphosphatase (Total ATPase)

Enzymes that transfer a phosphogroup from ATP to water are ATPases (Coll and Murphy, 1984) and are driven ion pumps. Within virtually all cells the sodium concentration is relatively low, while that of potassium ions is high. According to ion exchange theory, (Burger, et. al., 1996) the cytoplasm act as an analogous to an ion exchange resin with fixed charge in a lattice. Highly cross linked ion exchange resins exhibit specifically towards binding of certain ions.
One site of pesticide action is production of energy (Bloomquist, 2003). During oxidative phosphorylation high energy electrons traverse the mitochondrial electron transport chain, oxidizing reduced co-enzymes and finally producing water as they are transferred to oxygen. The built up protein motive force is used to drive the synthesis of ATP.

In the present study, the highest percentage of decrease 28.9% were observed in liver on the 90th day. As liver mitochondria is the chief source of ATPases and the liver is the chief detoxifying organ, most of the energy is utilized for the detoxification inorder to over come the stress induced by the endosulphan.

ATPase (Adenosine tri Phosphatase ) is hydrolysed to ADP and phosphate as the major energy source for muscle, brain and other tissues ATP is normally regenerated from ADP by oxidative phosphorylation. ATP is present as a complex with Mg²⁺ when aerobic metabolism fails to provide enough energy, extra ATP is produced during the anaerobic breakdown of glucose to lactic acid.

Activities of respiratory chain linked enzymes were inhibited at levels which corresponded to the concentrations of endosulphan used in vitro and it is an inhibitor of the electron transport chain. The invivo cytotoxic or insecticidal effects of endosulphan and its metabolites might therefore be the consequence of impaired mitochondrial bioenergetics. They affect mitochondrial oxidative phosphorylation and the respiratory chain which disrupt the ionic balance, membrane permeability and affect the active transport (Naqvi and Vaishnavi, 1993).
ADP reconversion to ATP can be blocked or partially blocked by some environmental contaminants and the translocator protein (TL) in the mitochondrial membrane can be chemically inhibited and is pH dependent. Changes in acid-base balance, magnesium status and the presence of abnormal metabolic products can have similar effects on xenobiotic inhibition of the translocator protein (Gorbach, et. al., 1965; Dalela, et. al., 1978; Lal, 1984; Wali, et. al., 1982; Kiran and Verma, 1988; Gupta, 1979; Narayan, et. al., 1984, Khanna, et. al., 1982).

Adenosine triphosphatases (ATPases) are involved in the maintenance of membrane permeability and energy production (Jowett, et. al., 1981). Total ATPase ($Na^+ K^+ \text{ATPase}$ and $Mg^{++} \text{ATPase}$) are both membrane bound and maintain ionic balance. They have been found to be affected by organochlorines (Dravyam and Rajamanickan, 2003; Ela, et. al., 1970; Leadam, et. al., 1974; Koch, et. al., 1972). Cytochrome oxidase being the terminal enzyme of the electron transport system was found to be the site of organochlorine action (Anderson, 1954).

It could be seen that endosulphan acting as an inhibitor of oxidative phosphorylation prevents ATP forming mechanism and there by blocks the utilization of oxygen that results in a decline in all states of respiration with isocitrate. Similar findings were reported from various studies (Tzagoloff, 1982; Koch, et. al., 1972; Desaiah, et. al., 1975a); Bhaskaran, 1988).

Many organochlorine pesticides inhibit ATPase adversely affecting osmoregulation and energy production. A great deal of information is available on the $in vivo$ and $in vitro$ effects of organochlorine pesticides on
ATPases with effects on both Na\(^+\) K\(^+\) and Mg\(^{++}\) ATPases varying with the tissues tested. The fish species used as well as with the pesticide (Desaiah and Koch, 1975a; Desaiah, et. al., 1975; Yap, et. al., 1972 Dalela, et. al., 1978; Verma, et. al., 1979; Sharma, 1988). ATPase thus considered as the target enzyme of organochlorine pesticide (Koch, 1969a; Davis and Wedmeyer, 1971; Akera, et. al., 1971; Narahashi and Hass, 1967; Matsmura and Narahashi, 1971; Desaiah, et. al., 1975, 1975; Dalela, et. al., 1978) ATPases are exclusively located in the plasma membrane (Lehninger, 1982). Lipid is a major component of the cell/plasma membrane regulating the activity of the membrane ATPases. In the present study, the lipid component in all assayed tissues found decreased. So this may also lead to inhibition of the ATPase activity. ATPase is sensitive to lipophilic inhibitors such as organochlorines like endosulphan, DDT, lindane etc. (Cutkomp, et. al., 1989). Organochlorine pesticides decrease the ATPase activity by forming an inhibitory complex with lipid component of the cell membrane (Reddy, et. al., 1991); Organochlorine like DDT, endosulphan, is capable of altering the transport of Na\(^+\) and K\(^+\) ions across the cell membrane and tissue osmolarity by inhibiting ionic balance (Narahashi and Hass 1967; Janicki and Kinter, 1971, Matsmura, 1975; Murphy, 1980).

Na\(^+\) K\(^+\) ATPase inhibition may lead to apoptosis or cell death, firstly by lowering the intracellular K\(^+\) which can affect mitochondrial function, by altering the membrane potential (Kashi and Ashraf 2000). Further oxidative stress due to (reactive oxygen species) – (ROS) could also lead to inhibition of Na\(^+\) K\(^+\) ATPase (Rodrigo, et. al., 2002). Histopathological study demonstrated the symptoms leads to cell death. Inhibition of ATPases enzyme occurs before
gross osmoregulatory dysfunction, which would point to the use of ATPase as an early warning of the pollutant induced damage to the ionic and osmoregulatory system. The ATPase system seems to be sensitive enzymatic bioindicator for pesticide (Reddy and Philip, 1994; Sancho, et. al., 1997; Joy, 1982; Srivastava, et. al., 1995). Thus, it can concluded that inhibition of ATPase activity by endosulphan may reduce ATP production (Racker, 1975).

5.6 Lactic Dehydrogenase and Succinic Dehydrogenase (LDH & SDH)

Cellular respiration is the set of reactions occurring within mitochondria of cells, that include the tricarboxylic acid cycle and electron transport. Pyruvate formed in the cytoplasm during glycolysis is transformed to utilizable energy stored in the form of ATP for further use in other cellular processes. Lactic dehydrogenase and succinic dehydrogenase are considered as markers of citric acid cycle and anaerobic respiration.

Lactic dehydrogenase is a tetrameric enzyme with perfect dihedral symmetry. LDH display nearly absolute specificity, transferring a proton into the “incorrect” pro-s-position no more than once in $5 \times 10^7$ catalytic cycles. This suggest a difference in transition state energies of about 40 KJ/mol for two isomers (Green, et. al., 1997).

Succinic dehydrogenase is one of the nine enzymes involved in tricarboxylic acid cycle and is the only one of the nine to be embedded in the inner membrane of the mitochondria. Succinic dehydrogenase catalyses the conversion of succinate to fumarate with the concomitant reduction of FAD to FADH$_2$ (Singer and Keamaey, 1956). Succinic dehydrogenase is a
flavoprotein (FAD) containing iron-sulfur centres (Singer and Keamaey, 1963).

Alterations in carbohydrate metabolism are produced due to the stress caused by endosulphan. Endosulphan and other organochlorine pesticides have the ability to disrupt the respiratory functions of the organisms (Evans, 1987). This leads to the development of internal hypoxic conditions which leads to anaerobic metabolism, indicated by the changes in the LDH and SDH activities.

Animal exposure to sublethal concentrations of a toxicant can produce stress and disturbances of the normal animal physiology. Decrease in the lactic acid, tissue glycogen and various other metabolic enzymes after exposure to endosulphan where reported (Hanke, et. al., 1983).

Lactate, a measure of anaerobic metabolism, has been widely used and increase of anaerobic metabolism have been shown to be a rapid and clear response against depletion of energy (Thillart and Smith, 1984). As in the case of LDH the binding affinity of endosulphan may be towards SDH than the enzyme substrate, so blocked productive complex hence the decline in metabolic activities.

Both these dehydrogenases are pivotal cellular metabolic enzymes between glycolytic pathway and tricarboxylic acid cycle. During the acute and subchronic exposure, elevation in LDH and SDH suggested that there is a shift in the respiratory metabolism from aerobic form to hypoxic anaerobic form following exposure to endosulphan. So, alterations observed in LDH and SDH activities suggested an impairment in carbohydrate metabolism in
the mice (Jayapratha, et. al., 1991; Reddy and Rao, 1991; Bhavan, et. al., 1997; Mishra and Shukla, 1997; Siddiqui, 1982; Konradt and Braunbeck; 2001).

The present study revealed that decreased SDH and LDH activities at the chronic exposure in the tissues of liver brain and kidney. Activities of the respiratory chain linked enzymes are inhibited at levels which corresponded to the concentration of endosulphan used in vitro. Both respiratory control ratio (RCR) and the ADP:O ratio fell sharply at endosulphan concentrations above 10 micrograms /ml (Dubey, et. al., 1984).

In the present study, the histopathological observation also clearly indicate the characteristic features of the cell death. Markers of the release of intracellular components such as the LDH detect the damage of outer cell membrane and are accepted as markers of cell death (Fernandez, et. al., 2006; Repetto, et. al., 2001; Sohn, et. al., 2004; Li and Zhang, 2002; Delescluse, et. al., 1998).

These marker enzymes mainly involved in the membrane transport (Fenoglio, et. al., 2005). Endosulphan and its metabolites interact with membrane lipids of sub cellular organelles like respiratory chain enzymes SDH and LDH and thereby affect the ion transport system (Gracia, et. al., 2003; Khan and Sinha 1993).

The results of present study indicated that endosulphan possess the dual properties of an uncoupler of oxidative phosphorylation and an inhibitor of electron transport chain. Endosulphan, being lipophilic, like other organochlorine pesticides (Murthy and Devi, 1982; Donker, 1992). It might be
interacting primarily with the mitochondrial lipoprotein surface resulting in structural damage and changes in the ionic permeability (Narahasi, 1971; Taskhi et. al., 2000)

The inhibition of mitochondrial respiration was found to occur in parallel to the inhibition of enzyme activities of the respiratory electron transport chain of mitochondria such as LDH and SDH thus the result of the present study indicates that the suppression of both the aerobic and anaerobic pathways of carbohydrate metabolism by the toxicant. And also it can be suggested that a limited supply of energy is ensured during pesticide toxic stress for the sustenance of vital activities of the animal through these pathways.

5.7 Acetyl Cholinesterase (Ach E)

One important neurotransmitter, acetylcholine is broken down by the enzyme acetylcholinesterase. It is a key enzyme of nervous system which is inactivated irreversibly by powerful organochlorine insecticide. Acetylcholine stimulates post synapses in the peripheral nervous system (PNS) and central nervous system (CNS) and at the neuromuscular junctions (Gracia, et. al., 2003). In organisms, messages are transmitted along the nerve cells using electrical impulses. When these reach the end of a nerve a chemical neurotransmitter activates the next cell in the chain (Duell, 2000). Acetylcholinesterase is specific for acetylcholine, a neurotransmitter that is released at many nerve synapses and neuromuscular junctions. The acetylcholine which is very toxic in excess must be destroyed rapidly to prepare synapse for transmission of another impulse.
Variation in acetyl cholinesterase and delayed neuropathy were reported due to the toxicity of organochlorine pesticides especially by endosulphan (Wilson, et. al., 1990; Fleming and Bardburg, 1981; Terril and Murphy, 1987; Ratner and Hoffman, 1984; Spassova, et. al., 2000; Garg, et. al., 2004; Kumar, 2003; Brorby and Beatty, 1987; Hoffman, 2000; Dalgard, 1984; Neemec, 1995, 1996 and Daeley and Hogan, 1980).

Based on the present study there is significant evidence that exposure of animals to the pesticide results in declination of overall biological activity, because acetyl cholinesterase is the enzyme responsible for breakdown of acetylcholine. Any process which eliminates this enzyme or inhibits its activity within the synapse would cause a marked increase in the accumulation of acetylcholine (Dutta and Arends, 2003). This would then, in effect, closely mimic the muscarinic acid and nicotinic receptor stimulation within the brain itself, therefore causing the overall decline in neural and muscular control (Hiran and Dane, 2003). The present study also revealed that the decreased movement and paralysis occurs during the chronic stage. The constant presence of acetylcholine within the neural gap keeps acetylcholine receptors consistently stimulated and will ultimately result in tetanic paralysis (Hiran and Dane, 2003; Kozlovskaya and Mayer, 1984). This may be the reason for paralysis.

It was found that endosulphan can cause quantifiable behavioural and physiological changes. These changes were potentially severe enough to disrupt the overall survivability of the animal. Along with reduced acetylcholinesterase activity nervous disorder was also seen. Clinical signs like restlessness, tremors, hypoactivated state, lethargy and paralysis have
been noticed. The inhibition of acetylcholinesterase which is responsible for the degradation of acetylcholine will result in excessive stimulation of cholinergic nerves, resulting in tremors and convulsions (Ferrari, et. al., 2004).

Due to inhibition of acetylcholinesterase, it was found that some behaviour disorders like food location, feeding, predator evasion, and escaping behaviour disturbed (Farri, 1977; Balint, et. al., 1995) Ferrari, et. al., 2004). Significant reduction of acetylcholinesterase activity were also reported by many investigators (Pavlov, et. al., 1992; Dutta, 1995; Walker and Thompson, 1991; Dutta, et. al., 1992; Richards and Datta, 1992; Ghosh and Bhattacharya, 1992; Reddy, et. al., 1991; Pan and Dutta, 1998; Reddy and Philip, 1994; Galgan and Bocquene, 1990; Antwi, 1987; Konradt and Braunbeck, 2001; Das and Mukherjee, 2003; Datta and Arends, 2003; Sevgiler, et. al., 2004).

The receptor sites like P. glycoprotein and MrP2 transporters in the brain capillary endothelial cells in the blood brain barrier which transports the xenobiotics from central nervous system to blood, disrupts the acetylcholinesterase activity. This may be also one of the major reason for the decrease of AchE (Dettbarn, et. al., 1999; Hassanein, 2002; Fernandez, et. al., 1999; Gopal, et. al., 1989; Sancho, et. al., 1998, Coppague and Matthews, 1974).

The neurobehavioural effects of endosulphan chiefly manifested in memory defect, partial aphasia, limited cognition and mental confusion (Paul, et. al., 1994). Inhibition of acetylcholinesterase activity and altered behavioural
pattern were reported by various animal models by several investigators (Dutta, 1995; Pan and Dutta, 1998; Hiran and Dane, 2003).

5.8 Histopathology

In the present study the histopathological changes in mice treated with endosulphan were observed with typical organochlorine toxicity and that was in a duration depended manner. Microscopic changes observed in different organs viz., brain, liver and kidney of treated group were noticed. Histopathological changes were studied to evaluate the extent of organ toxicity caused by endosulphan.

The brain is a highly complex organ which functions as a correlation centre. Due to the chemical stress induced by endosulphan, various pathological changes in nervous tissues were observed. In the present study, highest accumulation of endosulphan is noticed in brain tissues due to its high lipophilic nature.

After treatment with sublethal dose of endosulphan, clumping of the cells, and formation of multinucleated cells, shrinkage, lesions, vacuolation, acute haemorrhage, degenerative changes, separation of cell layers, splitting and irregular arrangement of cells were noticed.

Nerve cells of the brain with more than one nuclei and some clusters of nuclei were also observed. This may be due to hyper mitotic activity of neurons or neurotoxicity of the endosulphan suggesting enhancement of cell division by the toxicant (Lakomy, et. al., 1984; Haymaker, et. al., 1946).

Necrosis occurs as a result of the toxic effect to tissues by endosulphan and is a passive process associated with pathogenesis (Pileri, 1994; Jenner,

Following changes were noticed in the liver during acute, subchronic and chronic exposure. Occurrence of light hypertrophy, dialation of sinusoids, vacuolization of cell cytoplasm, disorganization of venacent ralis, amoebocytes, infiltration of lymphocytes, binucleated cells, haemorrhage, pycnotic state of nuclei, brown pigmentation, centrilobular enlargement, etc. All these reflects the cumulative effects of endosulphan in liver histology. They can be generally regarded as the induction, biotransformation and detoxification process occurs in liver (Hintan, et. al., 1978: Braunbeck, et. al., 1989; 1990, a,b,c). Some of these observations are characteristic in acute inflammatory process (Lajtner, et. al., 1996; Otludil, et. al., 2004; Johnalagadda and Rao, 1996; Klobucar, et. al., 1997) and pre-symptoms of cancerous conditions (Cotton, 1995; Young, 1992; Laster, 1998; Furie and Ramdolf, 1995).

Liver is the vital organ where the detoxification take place for endosulphan or any toxicant. The hepatic changes suggest mobilization of some kind of defensive mechanism in an endeavor to detoxify pesticides (Gill and Pande, 1991; Gill, et. al., 1988; Braunbeck and Appelbaum, 1999; Anuha, et. al., 1997 Amminikutty, et. al., 1977; Kumar and Pand, 1984; Eller, 1971; Gill, et. al., 1990).

One of the most affected organ by endosulphan is kidney. Lesions were comprised of intertubular haemorrhages, cystic dilation of tubules,
tubular degeneration, fatty changes which are indicative of nephrotoxicity (Gupta and Chandra, 1975; Cengiz, et. al., 2001). Histopathological alterations in the renal proximal tubules suggesting its mechanism in renal toxicity (Poovala, et. al., 1998; Smith, 1991, Gupta and Chandra 1977; Majumder, et. al., 1994). Cloudy swelling of the kidney is due to swelling the lining of tubular epithelium shows granular cytoplasm caused by the action of endosulphan (Cotton, 1995; Pileri, 1994; Jenner, 1994).

Thus, hisopathological changes seen in present study confirmed / indicated that exposure to sublethal concentration of endosulphan caused destructive and irrepairable damage to the body tissues. All these are the characteristics of reactions to the stress and chemical irritation induced by endosulphan (Triebskovn, et. al., 1998; Johnalagadda and Rao, 1996).

Presence of infiltration of lymphocytes and other cells are the signs of charges of lymphoid organs are indicative of toxicant induced alteration in immune functions. Necrosis, lesions, etc., are the signs of inbihitions of one or more essential metabolic process in the cells such as severe inhibition of RNA/DNA/Protein Synthesis/Lipid metabolism, etc., resulting from nuclear and E.R damage, disruption of mitochondrial energy generation and control, damage to lysosomes causing autolysis, depletion of ATP or drastic shift in the ionic balance of the body.

The peroxidation of lipids is a unique form of cellular injury and has been implicated in the genesis of necrosis. The hydrogen atoms on methyl carbons separating double bonds in polyonic fatty acids are highly susceptible to free radical attack. The abstraction of hydrogen atom from unsaturated
fatty acids during this attack yields free radicals of lipids and this represents the initiation of lipid peroxidation. Free radicals generated during the metabolism of various chemicals oxygen, hydroxyl radical, superoxide anion or same from perferryl ion have been suggested as initiators. The membraneous unsaturated fatty acids are obvious targets of lipid peroxidation resulting in the loss of structural integrity and function in the affected organelles. In addition to this localized damage, the break down products of lipid peroxides such as aldehydes migrate from their production site and may cause damage at distant loci. Inadequate supply of vital enzymes may be necrogenic. Present study also showed that there is depletion of lipid, protein and glycogen in assayed tissues due to the impact of endosulphan.

5.9 Residual Metabolites

Residual pesticides and other environmental contaminants with disrupting effects to body functioning are important environmentally because of their potential to cause deleterious effects in bio world. The problem with organochlorine pesticide is that they tend to persist in the environment, increasing exposure to wild life and humans (Forget, 1991; Chain, et. al., 1996; Dai, et. al., 2001; Erika, et. al., 2001).

The technical endosulphan given to mice contained Alpha and Beta isomers in the ratio 2:1. The Alpha isomer was assumed to be more stable and highly accumulated in the brain tissues after 90 days exposure. It is highly lipophilic also (Dai, et. al., 2001). In the present study after 21, days the alpha isomer is only detected in all tissues where, beta isomer and major metabolite endosulphan sulfate was not detected. On 60th day onwards, the beta
endosulphan is detected in all tissues. The major metabolite, endosulphan sulphate was not detected in brain tissues. In liver, it is seen as in moderate level after 60 and 90 days, whereas in kidney, it was detected only after 90 days of exposure. Although, most of the endosulphan is probably in the lipid fraction the neural action of endosulphan may depend upon its combination with non lipid components as well (Gupta, 1978).

Although, certain studies have found endosulphan sulfate to be the principal metabolite of endosulphan (Antonious et. al., 1998; Hansen and Goodman, 1980; Kinter and Forbis; 1983). Five days after a single oral administration C$^{14}$ labeled alpha endosulphan (2mg/kg body wt to female albino mice totals 75% 3 and 13% of the dose were eliminated in the faces and urine respectively. With the same dose of C$^{14}$ labelled beta endosulphan and under same condition, the values were 68% and 18.5%, respectively. Maximum residues of endosulphan which occurred in liver, brain and kidney (Devi, et. al., 1981, Gopal, et. al., 1980, Garg, et. al., 1980).

Endosulphan was metabolized in rats to endosulphan diol, endosulphan hydroxyl ethers endosulphan lactone, endosulphan sulfate and some unidentified polar metabolites (Dorough, et. al., 1987). Similar metabolites of endosulphan were identified in mice (Deema, et. al., 1999), alpha endosulphan, Beta endosulphan and endosulphan metabolites were detected in various studies (Brooke, 1984, Olafdottir, et. al., 1995, Verdoorn and Terblanche, 1995).

In the present study, brain shows the highest level of endosulphan concentration. Similar results were reported by various scientific studies.
Residues of 100-730 mg/kg have been documented for carcasses of Black
crowned neightheron and concentrations up to 1400 mg/kg were measured in
brain samples from the same species (Heinz, et. al., 1985; Lacher and
Goldstein, 1997) in fish (Kennish and Ruppel, 1997; Weimeyer, et. al., 1986).

All metabolic studies indicate that the parent compound was found to a
large degree in tissues and excreta. Similar conclusions can be drawn from the
work of Gupta and Gupta (1979) who found that almost half of the parent
compound was excreted unchanged in rabbits after endosulphan was injected
intravenously. The metabolites (Endosulphan sulfate, endosulphan diol) were
reported in tissues and excreta following longer exposure to endosulphan
(Deema, et. al., 1999; Drough, et. al., 1978). Based on the rapid appearance of
endosulphan sulfate in the liver following intravenous administration it may be
concluded that liver is the site of high metabolic activity in the conversion of
endosulphan to endosulphan sulphate (Khanna, et. al., 1989). In the present
study it was observed that liver has lowest concentration of endosulphan
sulphate and kidney and liver showed the lowest concentration of endosulphan
when compared to brain. More or less same trend also reported that male rats
fed 63 mg/kg body wt./day endosulphan over 30 days showed metabolism of
endosulphan in kidney. The endosulphan residues in kidney showed that
alpha endosulphan residues in kidney were quite high relative to levels in liver
and brain. Various endosulphan toxicity studies were reported in rats, mice
and rabbits (Gupta and Chandra, 1975; 1977, Gupta and Gupta 1977a,b).

The female mice were more susceptible to endosulphan toxicity
(Dikshith and Datta 1978; Agarwal, et. al., 1978). In the present study also
showed that total endosulphan residue was present in the brain tissue because
of its high lipophilic nature. By chronic conditions, degeneration, pigmentation, necrosis and variations in enzymes like increase in acid and alkaline phosphatase decreased production of ATPase, succinic dehydrogenase, lactic dehydrogenase, acetylcholinesterase. The metabolic system within the cell is irreparably damaged or disrupted i.e, the total collapse of metabolism. These reactions resulted in the accumulation of endoculphan in liver, kidney and brain.

Due to their lipophilic properties and resistance toward metabolic break-down, organochlorines are transported through the food chain with strong biomagnification at higher trophic levels (Van den berg, et. al., 1994). Further organochlorine, especially, the cyclodiene pesticides have also been implicated as the direct cause of death in several species of organisms (Sundolf, et. al., 1986; Olsen, et. al., 1993; Henriques, et. al., 1997). Quantifiable levels of this lipophilic xenobiotic and its metabolic products were also measured in various tissue in different species of organisms (Evans and Bouwman, 1993; Evans, 1995; Hothem, et. al., 1995; Olafsdottir, et. al., 1995; Custer, et. al., 1997; Goutner, et. al., 1997; Robertson and Boshaff 1986; Mundy, et. al., 1992).