WHOLE ANIMAL OXYGEN CONSUMPTION AND
HEPATOPANCREATIC TISSUE RESPIRATION

Introduction

Existence of living organisms depends on the ability of their cells to incorporate a number of simple compounds and transform them into more complex molecules required for cellular structure and function. The energy required for these synthetic reactions must be obtained from the same substrates by suitable oxidation reactions coupled to the generation of high energy phosphate esters. The entire process ultimately depends on the availability of molecular oxygen in cellular environment. Oxygen is made available to the tissues, and in turn to cells by respiration.

The total oxygen consumption of the animal reflects the basal metabolic status which reflects the general effect of several intrinsic and extrinsic environmental stresses. This serves not only as a tool in evaluating the susceptibility or resistance potentiality of the animal, but also useful to correlate the behaviour of the animal. General survey of literature showed that the pesticides affect the oxygen consumption both at the animal and tissue level (Ferguson and Goodyear, 1967; Lee, 1969; Eto, 1974; Silver, 1974; Koundinya and Ramamurthi, 1978; Rath and Misra, 1980; Kulkarni and Komath, 1980; Nagarathanamma and Ramamurthi, 1982; Bhagyalakshmi et al., 1983, 1984; Sreenivasula Reddy et al., 1986; Janardan Reddy et al., 1989, 1991, 1992). Hence the general analysis of oxygen consumption can be used as an indicator tc
evaluate the toxic potentiality of the chemical. The oxygen consumption at tissue level indicates the differential modulatory response and pattern of metabolic shift which is prevalent. These findings suggest the resistance of susceptibility of the tissues, which is important in toxicity evaluation. This type of programmed study also offers clue to the basic damage inflicted on the animal which could either increase or decrease the oxygen uptake. Such an event may lead to succession of chain reactions in metabolic pathways leading to activation or inhibition in the physiological functions of the animal.

Metabolic rate is usually estimated by the rate of oxygen consumption may fluctuate with season (Pandey, 1978) temperature (Hilali et al., 1979), size (Schmidt-Nielsen, 1970; Ramamurthi and Sainath Janak, 1973; Jagadish and Munshi, 1975; Jagadish and Singh, 1981) and sex (Ramamurthi and Sainath Janak, 1973). The metabolic rate also fluctuates with changes in rates of oxygen uptake, internal transport of tissue utilization of oxygen (Prosser, 1973).

Generally the basic mode of action of organophosphorus insecticide on animals of both invertebrates and vertebrates, is the disruption of nerve impulse transmission in the central and peripheral nervous system by inhibiting AChE, the enzyme that hydrolyzes the neurotransmitter, acetylcholine (O’ Brien, 1967, 1969; Aldridge, 1971; Fukuto, 1971; Brown, 1978; Rainsford, 1978). Inhibition of AChE by these pesticides was reported to cause abnormalities in some of the vital physiological processes like respiration and cardiovascular regulation (Eto, 1974; Silver, 1974). The organophosphorus pesticides are known to cause distress or failure of respiratory mechanism by affecting either respiratory centre in the brain or muscles involved in breathing (O’ Brien, 1967).

There were several reports showing OP insecticides, reducing whole animal and tissue respiration in fishes. Lee (1969) reported that methylparathion depressed respiration in gold fish. Ranke-Rybicke and Bozena (1975) reported that malathion, foshlor and dichlorvos inhibited oxygen uptake by 65%, 40% and 30% in Lebistes reticulatus. Koundinya and Ramamurthi (1978b) noticed that sumithion and sevin depressed respiration in the gill, liver, muscle, brain, intestine and kidney of T. mossambica. Sivaprasada Rao (1980) and Kabeer et al. (1981) noticed a diminution of oxygen consumption in T. mossambica exposed to methyl parathion and malathion. Sublethal concentrations of phosphamidon caused a decrease in the rate of $O_2$ uptake.
by the gill, liver, muscle and brain tissues of *T. mossambica* (Jayantha Rao, 1982). Nagaratnamma (1982) recorded a decline in whole animal respiration in *Cyprinus carpio*, upon exposure to malathion. Multiple sublethal concentrations of phosphamidon caused a decrease in O$_2$ uptake in *T. mossambica* and the magnitude of decrease was more pronounced upon chronic exposure (Jayantha Rao and Murthy, 1983). Rangaswamy (1984) reported that sublethal and lethal concentrations of endosulfan caused a decrease in whole animal oxygen consumption in *T. mossambica*. Dindale *et al.* (1982) recorded a decrease in oxygen consumption in rat when exposed to an OP compound, trialkyl phosphorodithioate. Respiratory disorders were recorded in rats on inhalation of an OP gas, diazomethane (Stain *et al.*, 1983).

Sitkiewiez *et al.* (1976) noticed inhibition of respiration in liver homogenates of rats upon dipterex and DDVP intoxication. Spetale *et al.* (1977) reported that OP compounds like parathion, malathion and dimethoate, when added to the incubation medium containing normal rat liver mitochondria, decreased state 3 (fast rate) and state 4 (slow rate) respiration in mitochondria in the presence of glutamate and malate. Similarly, malathion has been reported to reduce oxygen consumption of liver and kidney slices of hen (Gupta *et al.*, 1974). Zimmerman and Rapoport (1982) reported that ouabain caused a decrease in oxygen consumption to the tune of 12% and 45% in reticulocytes and slices of kidney cortex respectively. Janardan Reddy *et al.* (1992) noticed a decrease in whole animal oxygen consumption and kidney respiration in multiple sublethal doses of phosalone in rat.

Very few attempts have been made on the effect of different OP compounds on respiration of different species of crabs. Exposure of crabs like *Barytelphusa cunicularis* (Nagabhushanam and Diwan, 1972), *Paratelphusa jacquimontii* (Kulkarni and Kameth, 1980), *Oziotelphusa senex senex* (Bhagyalakshmi, 1981) to OP compounds inhibited the whole animal oxygen consumption. A glance at the available literature indicates the fact that the OP insecticide on whole animal oxygen consumption and tissue respiration in crustaceans especially in crabs has not been studied as thoroughly as it should have been. This assumes further significance in the light of the fact that respiration has been identified as an indicator of response to sublethal stress in organisms exposed to toxic substances (Anderson, 1971; Sprakge, 1971; Waldichuck, 1979; Hughes, 1981). Hence an attempt was made to study the effects of sublethal concentration of phosalone for the period of 1, 3 and 7 days on
whole animal oxygen consumption and hepatopancreatic tissue respiration of freshwater field crab, *O. senex senex*.

**Results and Discussion**

**Whole animal oxygen consumption**

The rates of whole animal oxygen consumption and hepatopancreatic tissue respiration of control and exposed crabs to sublethal concentrations of phosalone for 1, 3 and 7 days and also recovery span for 1, 3 and 7 days are presented in Table 3.1 and in figure 3.1. The sublethal concentration of phosalone caused a gradual and significant decrease (P < 0.05) in whole animal oxygen consumption (Table 3.1). The inhibition of oxygen uptake increases progressively as the time course study reveals for 1 day (7.54%), 3 day (16.10%) and 7 day (30.29%). The sublethal concentration of phosalone also caused a gradual and significant decrease (P < 0.05) in hepatopancreatic tissue respiration on 1, 3 and 7 days. The per cent diminution of oxygen consumption of hepatopancreas is in the following order: 1 day (11.63%), 3 day (22.76%) and 7 day (32.24%). There is very little information available on the whole animal and tissue respiration of invertebrates in general and crustaceans in particular in relation to OP pesticide treatment. Decrease in the rate of oxygen consumption was reported by several workers in different species of fish exposed to different OP compounds. Lee (1969) observed respiratory distress in gold-fish exposed to methyl parathion. Exposure of fish, *Labistes reticulatus* to 3.4 mg malathion inhibited respiration by 6.5%, for the same concentration the per cent inhibition by foschlor was 40% and by dichlorvos the inhibition was 36% (Ranke-Rybica and Bonzene, 1975).
Table 3.1: Effect of sublethal concentration of phosalone on whole animal oxygen consumption (μl O₂/h) and hepatopancreatic tissue respiration (g wt/O₂/h) of O. senex senex. Values are mean ± SD of 6 individual observations. Values are significant at P < 0.05.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Exposure (days)</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1D</td>
<td>3D</td>
</tr>
<tr>
<td>Whole animal oxygen</td>
<td>3.84</td>
<td>3.55</td>
<td>3.22</td>
</tr>
<tr>
<td>consumption</td>
<td>±0.22</td>
<td>±0.25</td>
<td>±0.25</td>
</tr>
<tr>
<td>% Change</td>
<td>-</td>
<td>-7.54</td>
<td>-16.10</td>
</tr>
<tr>
<td>Hepatopancreatic</td>
<td>1.96</td>
<td>1.73</td>
<td>1.51</td>
</tr>
<tr>
<td>respiration</td>
<td>±0.12</td>
<td>±0.13</td>
<td>±0.09</td>
</tr>
<tr>
<td>% Change</td>
<td>-</td>
<td>-11.63</td>
<td>-22.76</td>
</tr>
</tbody>
</table>

NS: Not significant at 0.05 level.

![Bar chart showing percentage change in WAOC and HTR](image)

Fig.3.1: Effect of sublethal concentrations of phosalone on whole animal oxygen consumption (WAOC) and hepatopancreatic tissue respiration (HTR) of O. senex senex

Exposure of crabs *Barytelphusa cunicularis* (Nagabhushanam and Diwan, 1972); *Paratelphusa jacquemontii* (Kulkarni and Kamath, 1980), *Oziotelphusa senex senex* (Bhagyalakshmi, 1981) to OP compounds inhibited the whole animal oxygen consumption. Progressive decline in oxygen uptake was observed after 1 hour
(20.15%), 6 hours (23.14%), 12 hours (36.46%) and 24 hours (55.31%) in crab, *O. senex senex* exposed to 0.6 mg/l sumithion i.e. LC$_{50}$/24 hours (Bhagyalakshmi, 1981). In the same species, 0.4 mg/l sumithion i.e. LC$_{50}$/48 hours caused 33.14% and 40.67% reduction in oxygen uptake after 24 and 48 hours exposure respectively (Bhagyalakshmi, 1981). Sublethal concentration of sumithion (0.1 mg/l) also suppressed the respiratory rate to 13.93%, 28.15%, 37.96% and 40.67% after 1, 3 and 7 days respectively. Similar trend was reported in fish, *Cyprinus carpio*, exposed to sublethal concentration of methylparathion for 7, 15 and 30 days (Nagarathnamma, 1981). In addition, several authors have observed diminution in oxygen consumption of different fishes acutely exposed to sublethal and lethal concentrations of different OC and OP onsecticides (Uthaman, 1977; Sivapradasa Rao, 1980; Madhu, 1983; Sambasiva Rao, 1984; Radhaiah, 1988; Janardan Reddy *et al.*, 1991). Which has been attributed the insecticide stress and gill damage.

In order for gases to pass between the environment and the blood the first requirement is a thin membrane, covering the gill. In order for the process of diffusion to occur a higher concentration of oxygen and a lower concentration of carbon dioxide must be maintained outside the membrane than in the blood of the respiratory membrane. Therefore, the secondary requirements for a respiratory system is a ventilation mechanism to move the respiratory medium (water or air) past the respiratory membrane (Webster and Webster, 1974). In crustaceans this is accomplished by sustained vibrating movements of the scaphognathite of maxillae and exopodites of maxillepeds, which create a constant water current passing over the gills. Apparently, vibrating movements of scaphognathite and exopodites are produced by a set of muscles which are under nervous control (Hyman, 1959; Prosser, 1973). Derangement in the rhythmic movements of these organs as a results of AChE inhibition could lead to a decrease in the rate of water flow over gill surface and hence in the amount of dissolved oxygen available for diffusion. AChE inhibitory activity of OP insecticides, including phosalone has been demonstrated (FAO/WHO, 1973b; Bhagyalakshmi and Ramamurthi, 1980; Silas Ebenezar 1981; Palanivelu, 1984; Bhagyalakshmi *et al.*, 1984, 1985; Ravi and Selvarajan, 1986; Bulusu Saraswathi and Indira, 1986; Sunita *et al.*, 1987; Janardan Reddy, 1988). The above observations suggest that phosalone through AChE inhibition, might have disrupted the rhythmic movements of the scaphognathite and exopodites of *O. senex senex* causing a decrease in whole animal oxygen consumption.
It is well known that maintenance of structural integrity of the cells of respiratory organs is essential for respiration to occur at normal rate. As such any factor that causes a damage to the structural integrity of the cells of respiratory organs like gills, may adversely affect the process of respiration leading to a decrease in oxygen consumption since several of the OP insecticides were known to cause cellular lesions in respiratory organs (Dikshit et al., 1978; Mathur, 1979; Mathur and Rane, 1979; Nagarathnamma, 1982; Bulusu Saraswathi and Indira, 1986) particularly in crabs (Jayantha Rao, 1983; Bhagyalakshmi, 1981). The above observations lend credence to the suggestion that phosalone, an organophosphorus pesticide, might cause gill damage, resulting in a decrease in whole animal oxygen consumption of *O. senex senex*. Another possible factor that could be implicated in causing decrease in oxygen consumption of crabs exposed to sublethal concentrations of phosalone is the formation of a 'mucus film' over gill surface which is likely to affect respiratory rate by reducing the effective surface area of diffusion and the rate at which diffusion occurs. Formation of a thin mucus film over the gill surface has also been observed in fishes (Koundinya and Ramamurthi, 1979; Nagaratnamma, 1981) and crabs (Bhagyalakshmi, 1981) exposed to different OP pesticides. Such a phenomenon has also been observed in the present study which may account for the decrease in whole animal oxygen consumption. Further it has been observed that mucus deposition over gill surface was greater on day 7 than on the preceding days and this, perhaps, is responsible for a greater decrease in whole animal oxygen consumption on day 7.

These observations suggest that decrease in whole animal oxygen consumption of *O. senex senex* could be due to the combined effect of the factors explained above resulting in respiratory distress. Evidence to show that respiratory rate diminution is one of the main symptoms of insecticide toxicity in crustaceans is unequivocal (Cantelmo et al., 1978; Sharp et al., 1979; Ranga Rao, 1979).

**Hepatopancreatic tissue respiration**

Table 3.1 presents results on oxygen consumption of hepatopancreas of *O. senex senex* exposed for 1, 3 and 7 days to sublethal concentration of phosalone. The above results showed that there was a gradual and significant decrease in hepatopancreatic tissue respiration (Figure 3.1) and the maximum inhibition was observed on day 7 (Table 3.1; \( P < 0.05 \)).
A large number of investigations pertaining to the effects of different insecticides on oxygen consumption of the hepatopancreas of invertebrates and liver in the vertebrates are available. Sitkieweiz et al. (1976) noticed inhibition of respiration in liver homogenates of rats upon dipterex and DDVP intoxication. Spetale et al. (1977) reported that parathion, malathion and dimethoate, when added to the incubation medium containing normal rat liver mitochondria, decreased state 3 (fast rate) and state 4 (slow rate) respiration in mitochondria in the presence of glutamate and malate. Similarly Gupta et al. (1974) noticed that malathion caused reduction in oxygen consumption of liver and kidney slices of hen. Zimmerman and Rapoport (1982) reported that ouabine caused decrease in oxygen consumption of reticulocytes and slices of kidney cortex respiration. Bhagyalakshmi (1981) and Basha et al. (1984) reported decrease in hepatopancreas and liver respiration of O. senex senex and T. mossambica exposed to sublethal concentration of sumithion and lindane respectively. Sreenivasula Reddy and Ramana Rao (1985) observed a decrease in hepatopancreatic tissue respiration of marine crab, Scylla serrata, exposed to sublethal concentrations of phosphamidon. Rangaswamy (1984) reported decrease in oxygen consumption of the liver of T. mossambica chronically exposed to sublethal concentrations of endosulfan. Srinivasa Moorthy (1983) reported decrease in hepatopancreatic tissue respiration of Lamellidens marginalis, a freshwater mussel, exposed to sublethal and lethal concentrations of methyl parathion. Satyaprasad (1983) observed diminution in oxygen uptake of the liver of T. mossambica, chronically exposed to multiple sublethal concentration of lindane. Rajeswari (1989) reported that endosulfan caused decrease in hepatopancreatic tissue respiration in O. senex senex. These authors have suggested that decrease in oxygen intake might be due to histopathological damage or necrosis of the hepatopancreas and liver. Several authors have reported damage to the hepatopancreas or liver of aquatic and terrestrial animals treated with different OP and OC insecticides. Konar (1977) reported degenerative changes in liver cells of Labeo rohita and Heteropneustes fossilis exposed to sublethal concentration of heptachlor. Amminikutty and Rege (1977) observed necrosis in the liver of the fish, Gymnocarymbus ternetzi, exposed to thiodon. Kennedy et al. (1970) observed histopathological damage to the liver of fish treated with methoxychlor. Jayantha Rao (1982) reported that the occurrence of rupture and vacuolation in the hepatocytes of the liver of T. mossambica exposed to phosphamidon. After absorption from the intestinal tract, most of the incoming nutrients will be passed directly to the hepatopancreas/liver, a major centre which
assists in digestion and controls a great deal of the metabolism of the body by its selective filtration and secretion. Further, the hepatopancreas/liver acts as a detoxifying organ by removing the toxic materials from the blood and detoxify them. Edwards (1973) reported that the insecticides will be accumulated to a greater extent in the hepatopancreas/liver than in the other tissues of the biosystem. Brown (1963) observed that the hepatopancreas/liver could be badly damaged during pesticide treatment. The above observations indicate that the hepatopancreas/liver is actively involved in nutrient metabolism as well as in insecticide metabolism. The above observations suggest that sublethal concentrations of phosalone might have caused histopathological damage/lesions to the hepatopancreas of *O. senex senex* resulting to a gradual decrease in its respiration or oxygen uptake for 1, 3 and 7 days. Edwards (1973) reported that there was a greater decrease in oxygen consumption of the hepatopancreas/liver than other tissues, since the hepatopancreas is the major centre for pesticide metabolism. In general, a decrease in whole animal oxygen consumption leads to a decrease in oxygen consumption of the body tissues (Prosser, 1973) and this perhaps, explains the observed decrease in hepatopancreatic respiration in crabs in the present investigation.

During the recovery period the inhibition trend in whole animal oxygen consumption and hepatopancreatic tissue respiration was gradually reversed near to normal level by day 7, indicating that the animal gradually recovered when they were transferred from the phosalone containing water into normal water. The observed per cent recovery in crabs was for 1 day (23.94%), 3 day (12.20%) and 7 day (2.55%) in whole animal oxygen consumption and for 1 day (24.18%), 3 day (13.57%) and 7 day (2.75%) in hepatopancreatic tissue respiration. The above results indicate that the animal recovers during depuration time by reversing the patterns in rhythmic movements of the scaphognathite and exopodites, structural integrity of gills, mucus film on gills and histopathological damage or necrosis of gills of crab, when they are transferred to pesticide free medium.
ENZYMES RELATED TO ENERGY METABOLISM

Introduction

Living organisms require continuous supply of energy for the build up and maintenance of their organisation. The energy will be released by the oxidation of the foodstuffs like carbohydrates, fats and proteins. Energy thus liberated during the oxidation is trapped in phosphate bonds in the form of ATP (Adenosine triphosphate). Oxidations of food stuffs and synthesis of generated energy in the form of ATP are the two important aspects of energy metabolism which occur through a series of metabolic steps governed by many enzymes. Efficiency of organism to carry out its metabolic activities is solely dependent on the activities of enzymes concerning energy production and any alteration in these enzyme activities would influence the bioenergetic demands of the organism.

Enzymatic studies are essential to understand metabolic functions of a cell, since these are the ubiquitous biochemical reactions necessary for various functional activities of the cell and support the synthesis of material upon which the cell depends for maintenance of structure, growth and multiplication. Krebs cycle is the final common pathway for the oxidation of carbohydrates, lipids and proteins, since glucose, fatty acids and many aminoacids are all metabolized to acetyl Co-A. As the acetyl Co-A is oxidised to carbon dioxide and water through a series of metabolic steps, reducing equivalents in the form of electrons are released as a result of the activity of specific dehydrogenases leading to the generation of high energy phosphate
bonds through oxidative phosphorylation. The energy thus generated is of prime importance for the animal and any variation in or inhibition of the activity levels of the enzymes of the citric acid cycle, may therefore disturb the proceedings of the entire process causing many complications of variable nature.

Eventhough the pesticides are thought to interfere at various levels in the above process, very little information is available on the effects of pesticides on energy metabolism. In fact the present investigation is an attempt to understand the effects of sublethal concentration of phosalone on some selected enzymes related to energy metabolism. Succinate dehydrogenase (SDH) which catalyses the dehydrogenation of succinate to fumarate and ICDH, which catalyses the dehydrogenation of isocitrate to $\alpha$-ketoglutarate, are the two important enzymes of the citric acid cycle. Lactate dehydrogenase (LDH) is the representative of anaerobic metabolism. Glucose-6-phosphate dehydrogenase (G-6-PDH), a key enzyme that is involved in Hexose monophosphate (HMP) pathway. Any biosystem utilizes the energy by hydrolyzing the phosphate bond of ATP that involves in the cleavage of ATP to ADP/AMP and inorganic phosphate with energy release. SDH, LDH and G-6-PDH represent the energy generating system while ATPase represents the energy utilization and study of these key enzymes during pesticide intoxication would give a clear cut picture about the bioenergetic requirements of an organism during pesticide treatment.

A large body of literature is available on the effects of different insecticides on SDH, ICDH, G-6-PDH, LDH and ATPase activities in different non-target organisms. Desaiiah and Koch (1975b) and Verma et al. (1979) reported decrease in the activities of total, Mg$^{2+}$, Na$^+$-K$^+$ ATPases and G-6-PDH in liver, gill and muscle of fishes Ictalurus punctatus with toxaphene and L. rohita and S. fossilis with endosulfan respectively. Desaiiah and Koch (1975c) reported decrease in Mg$^{2+}$ and Na$^+$-K$^+$ ATPases in liver, gill and muscle of fish, H. fossilis exposed to aldrin. Dalela et al. (1978) observed diminution in Mg$^{2+}$ and Na$^+$-K$^+$ ATPases in gill, liver and muscle of Channa punctatus exposed to DDT. Sharma et al. (1979) reported that endrin depressed SDH activity in liver, gill and muscle of fish O. punctatus. Rhead et al. (1981) observed a diminution in total, Mg$^{2+}$ and Na$^+$-K$^+$ ATPases in hepatopancreas, gill and claw muscle of Carcinus maenus under DDT treatment. Dhoerty et al. (1981) have found decrease in Mg$^{2+}$ and Na$^+$-K$^+$ ATPases in muscle
and hepatopancreas of lobster, *Astacus fluriatilis*, exposed to aldrin and dieldrin. Sastry and Siddiqui (1983) noticed inhibition in SDH activity and elevation in LDH activity in liver of *Channa punctatus* upon endosulfan intoxication. Rangaswamy (1984), Rajendra Prasad Naidu *et al.* (1986) reported decrease in ICDH and SDH activities while increase in LDH and G-6-PDH activities of hepatopancreas, gill and claw muscle of *Oziotelphusa senex senex* exposed to endosulfan. Lakshmi (1984) and Vasanthy and Rangaswamy (1987) observed a decrease in SDH activity of the liver and muscle of *S. mossambicus* exposed to lindane and thiodon respectively. Kalarani *et al.* (1984) reported a marked decrease in ICDH and SDH activities and increase in LDH and G-6-PDH activities of *Pila globosa* exposed to endosulfan. Satyaprasad (1983) reported an increase in G-6-PDH activity of the liver, gill and muscle of *T. mossambica* exposed to lindane. Rajeswari (1989) observed decrease in total, Mg$^{2+}$, Na$^+$ -K$^+$ ATPases and increase G-6-PDH activity in hepatopancreas, gill and claw muscle of *O. senex senex* exposed to endosulfan over a 30 day exposure period. Sreenivasulu Reddy *et al.* (1988) noticed a significant decrease SDH activity in gill, muscle and midgut gland of marine prawn *Penaeus indicus* exposed to phoshamidon.

Very few attempts have been made on the effect of different OP pesticides on ICDH, SDH, LDH, G-6-PDH and ATPases of different crabs. Bhagyalakshmi (1981) observed a marked decrease in ICDH and LDH activities, while increase in LDH and G-6-PDH activities of freshwater crab, *O. senex senex* exposed to sumithion. Bhagyalakshmi *et al.* (1984) reported that sumithion caused decrease in the activity levels of SDH and MDH and increase in LDH in hepatopancreas of crab. Bhagyalakshmi *et al.* (1984b) also reported that sumithion also caused decrease of SDH and ICDH activities and increase of LDH activity in hepatopancreas of crab over a 7 day exposure period. Sreenivasulu Reddy *et al.* (1982, 1983) observed a significant decrease in SDH activity and increase in LDH activity in hepatopancreas and claw muscle of sumithion treated crab respectively. Methyl parathion also caused an increase in LDH activity and decrease in SDH activity in claw muscle of crab (Sreenivasulu Reddy *et al.*, 1986).

The above review of literature indicates that there very few reports relating to the action OP pesticides particularly phosalone on enzymes related to energy metabolism in invertebrates particularly in crabs. This assumes further significance in the light of the fact that the studies of enzymes related to energy metabolism justify
the alterations observed in whole oxygen consumption and tissue respiration (Chapter III, Part A) of crab exposed to sublethal concentration of phosalone. Hence the present study was carried out.

Results and Discussion

Table 3.1 presents the results of activity levels of ICDH and SDH in the hepatopancreas of _O. senex senex_ exposed 1, 3 and 7 days to multiple sublethal concentration of phosalone. The results show that there was a decrease in ICDH and SDH activities in hepatopancreas (Fig. 3.2) and the maximum decreases in ICDH (41.81%) and SDH (19.69%) activities was found on day 7 (P).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Exposure (days)</th>
<th>Recovery (days)</th>
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<td>1D</td>
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<tr>
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<tr>
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<tr>
<td>% Change</td>
<td>-13.68</td>
<td>-22.29</td>
<td>-29.12</td>
</tr>
</tbody>
</table>

NS: Not significant at 0.05 level.

Not much literature is available on the effects of different insecticides on tissue ICDH and SDH activities in various animals. Sharma _et al._ (1979) reported that a considerable decrease was observed in SDH activity in the liver, gill and muscle of the fish _Ophioccephalus punctatus_, under sublethal concentration of endrin toxicity. Bhagyalakshmi (1981) reported a marked decrease in ICDH and SDH activities of the hepatopancreas, gill and claw muscle of _O. senex senex_ exposed to sublethal concentration of sumithion. Sastry and Siddiqui (1983) reported a gradual decrease
in SDH activity of the liver of *Channa punctatus* exposed to low concentration of endosulfan. Kalarani *et al.* (1984) reported a marked decrease in ICDH and SDH activities of the hepatopancreas, gill and foot muscle of *Pila globosa* exposed to sublethal concentration of endosulfan. Lakshmi (1984) and Vasanthy and Rangaswamy (1987) observed a significant decrease in SDH activity of the liver and muscle of *S. mossambicus* exposed to sublethal concentration of lindane and thiodon respectively. Rangaswamy (1984) observed a significant decrease in ICDH and SDH activities of the liver, gill and muscle of *T. mossambica* exposed to sublethal and lethal concentration of endosulfan. Rajendraprasad Naidu *et al.* (1986) observed considerable decrease in ICDH and SDH activities of the hepatopancreas, gill and claw muscle of *O. senex senex* exposed to sublethal concentration of endosulfan. Azhar Baig (1988) reported marked decrease in SDH activity of the muscle of *C. punctatus* exposed to sublethal concentration of heptachlor.

Inhibition of SDH activity has been reported during liver disease (Nairo *et al.*, 1973). The inhibitory effect of OP pesticide sumithion on SDH and ICDH activity points to the degree of disturbances of mitochondrial integrity (Bhagyalakshmi, 1981). It has been reported that treatment of organophosphorus insecticide alters the mitochondrial structure (Miroslow, 1973). In several animals like fish (Koundinya and

Krebs cycle is the final common pathway for the oxidation of carbohydrate, lipid or protein, since glucose, fatty acids and amino acids are all metabolized to acetyl-COA which is oxidized to carbon dioxide and water through a series of metabolic cascades. Dehydrogenases release electrons from the substrates of the citric acid cycle. ICDH catalyzes the dehydrogenation of isocitrate to -ketoglutarate and SDH catalyses succinate to fumarate. The electrons thus released pass through the electron transport chain producing high energy phosphate bonds (ATP) and ultimately combine with molecular oxygen to form water. Hence the availability of molecular oxygen is useful for normal functioning of the citric acid cycle and any disturbance in the form of non-availability of oxygen or decrease of oxygen consumption could lead to decrease in the activities of dehydrogenases. The above observation suggests that decrease in ICDH and SDH could be attributed to hypoxic condition occurred in the biosystem of crab under the treatment of sublethal concentration of phosalone, which was indicated by decrease in whole animal oxygen consumption (Figure 3.1) and hepatopancreatic tissue respiration (Figure 3.1) in phosalone treated crab. The other important factor is the relationship of ICDH and SDH activities to the mitochondrial integrity, since, ICDH and SDH are known for their exclusive mitochondrial localization (Harper, 1981). A few authors have reported a role of insecticides in causing alterations in mitochondrial structure and in the activity of associated enzymes indicating that the multiple sublethal concentration of insecticides caused disruption of structural integrity of mitochondria. Bhagyalakshmi (1981) who reported decreased SDH and ICDH activities suggested a inhibited mitochondrial oxidation of their corresponding substrates which may lead to a drop in the production of energy. These observations suggest that sublethal concentration of phosalone induce hypoxia which is perhaps, responsible for the decrease in ICDH and SDH activities of the hepatopancreas O. senex senex. However, further studies need to be conducted in this regard before any definite conclusions are drawn.
Total adenosine triphosphatases (ATPases)

Table 3.2 presents the results on total ATPase activity levels in the hepatopancreas of *O. senex senex* exposed for 1, 3 and 7 days at sublethal concentration phosalone. The results show that there was a gradual decrease in total ATPase activity (Figure 3.2) and the percent decrease of 13.68 for 1 day 22.29 foror 3 day and 29.12 for 7 day (P < 0.05) was observed.

ATPases are a set of complex enzyme systems that play an important role in the maintenance of permeability properties of cell membranes. Depending on the specific ion requirement ATPases are called as Mg$^{2+}$ ATPase, Na$^{+}$-K$^{+}$ ATPase and Ca$^{2+}$ ATPase. They are associated with intracellular molecules exist in myosin, actomyosin, mitochondria, microsomes are well known that the ATPases are of plasma membrane (Lehninger, 1982). One of the major components of the plasma membrane, the lipid, is represented by phospholipid, cholesterol and cephalin (Cohn et al., 1976). The lipid component of the phospholipid fraction, has an important role in regulating the activity of membrane bound ATPases. Mitochondrial ATPase functions as a coupling factor in oxidative phosphorylation (Pullman et al., 1960; Renefsky et al., 1960; Renefsky and Warner, 1965). Several authors have reported decrease in the activity levels of tissue ATPase in different non-target species exposed to organochlorine insecticides. Desaiiah and Koch (1975b) reported decrease in total, Mg$^{2+}$ and Na$^{+}$-K$^{+}$ ATPases in the liver, gill and muscle of the fish, *Ictalurus punctatus*, exposed to toxaphene. Verma et al. (1979) observed decrease in total, Mg$^{2+}$ and Na$^{+}$-K$^{+}$ ATPases in the liver, gill and muscle of *Labeo rohita* and *Saccobranchus fossilis*, exposed to sublethal concentration of chlordane. Dalela et al. (1978) observed diminution in Mg$^{2+}$ and Na$^{+}$-K$^{+}$ ATPases in the liver, gill and muscle of *Channa gachua* exposed to sublethal concentrations of endosulfan. Rhead et al. (1981) reported decrease in total, Mg$^{2+}$ and Na$^{+}$-K$^{+}$ ATPases in the hepatopancreas, gill and claw muscle of *Carcinus maenus* exposed to DDT intoxication. Doherty et al. (1981) observed decrease in Mg$^{2+}$ and Na$^{+}$-K$^{+}$ ATPases in the hepatopancreas and muscle of lobster, *Astacus fluviatilis*, exposed to low concentrations of aldrin and dieldrin. Desaiiah and Koch (1975c) observed diminution in Mg$^{2+}$ and Na$^{+}$-K$^{+}$ ATPases in the liver, gill and muscle of the fish, *H. fossilis*, exposed low concentrations of aldrin. Cutkomp et al. (1976) reported decrease in Mg$^{2+}$ ATPases activity in the liver and muscle of fish exposed to sublethal
concentrations of DDT. Rajeswari (1989) observed a diminution in total, Na\(^+\)-K\(^+\) ATPase and Mg\(^{2+}\) ATPases in hepatopancreas gill and claw muscle of *O. senex senex* exposed to sublethal concentration of endosulfan over a 30 day period. Rajendraprasad Naidu (1989) reported a decrease in Mg\(^{2+}\) and Na\(^+\)-K\(^+\) ATPases activities in *O. senex senex* exposed to sublethal and lethal concentrations of endosulfan. The present investigation showed a significant decrease (P < 0.05) in the ATPase activity in hepatopancreas of phosalone treated crabs (Fig.3.2). A similar diminution of the chicken spinal cord ATPase activity during OP insecticides poisoning was reported by Brown and Sharma (1976). Hoskin *et al.* (1969) reported irreversible inhibitory effect of OP insecticide on axonal conduction to be dependent on concentration and time and proposed the observed effects to be due to the binding of these compounds to an unspecified membrane component. Dikshith *et al.* (1978) reported a decrease in the ATPase activity following parathion administration to rat.

Since Na\(^+\)-K\(^+\) ATPases could maintain the transmembrane ionic gradients (Skou, 1957), the secondary involvement of this gradient in amino acid and sugar transport (Eddy, 1968), its inhibition by the OP compounds could result in altering the properties of membrane and thus block the active transport phenomenon. The decreased ATPase activity might be due to structural changes of the mitochondrial membranes (Miroslow, 1973). Since the total ATPase comprising of Na\(^+\)-K\(^+\) ATPase and Mg\(^{2+}\) ATPase, decreased in all tissues of phosalone treated crabs, it is quite likely that the basic cellular functions like active transport phenomenon, oxidative phosphorylation are also likely to be decreased leading to loss of ions (Tables 2.5, 2.6; Fig. 2.5, 2.6) depression in respiratory rate and decrease in activities of TCA cycle enzymes (Table 3.2 & 3.3).

Lactate dehydrogenase (LDH)

Table 3.2 presents the results on LDH activity of the hepatopancreas of *O. senex senex* exposed to 1, 3 and 7 days to sublethal concentration of phosalone and also the changes in recovery span for 1, 3 and 7 days after transfer from pesticide water to normal freshwater. The results show that there was a progressive increase in LDH activity of hepatopancreas (Figures 3.3) and the percent increase for 1 day (19.14%), 3 day (42.55%) and 7 day (53.19%) observed being significant (P < 0.05). LDH catalyses the interconversion of lactate to pyruvate. This is dependent on a heterogenous group of components, called as isoenzymes, which show significant
quantitative changes during certain pathological conditions (Hoar et al., 1970; Harper et al., 1989).

Table 3.3: Effect of sublethal concentration of phosalone on LDH and G-6-PDH (μ moles formazan/mg protein/h) in hepatopancreas of crab, O. senex senex. Values are mean ± S.D. of 6 individual observations. Values are significant at P < 0.05.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Exposure (days)</th>
<th>Recovery (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1D</td>
<td>3D</td>
<td>7D</td>
</tr>
<tr>
<td>LDH</td>
<td>0.09</td>
<td>±0.01</td>
<td>±0.02</td>
</tr>
<tr>
<td>% Change</td>
<td>-</td>
<td>19.14</td>
<td>42.55</td>
</tr>
<tr>
<td>G-6-PDH</td>
<td>0.07</td>
<td>±0.008</td>
<td>±0.006</td>
</tr>
<tr>
<td>% Change</td>
<td>-</td>
<td>13.84</td>
<td>30.76</td>
</tr>
</tbody>
</table>

NS: Not significant at 0.05 level.

Several authors have studied the effect of different insecticides on tissue LDH activity of different non-target organisms. It has been reported that sumithion increases LDH activity in rats (Lasota et al., 1973); fishes (Koundinya and Ramamurthi, 1978a; Sivaprasada Rao and Ramana Rao, 1979); snails (Ramana Rao and Ramamurthi, 1978) and earthworms (Dayananda Reddy, 1980). Bhagyalakshmi (1981) reported elevated levels of LDH in the hepatopancreas, gill and claw muscle of O. senex senex repeatedly treated with multiple sublethal concentrations of sumithion. Natrajran (1981) observed increase in LDH activity of the gill and muscle of Channa straitus treated with metasystox. Satyaparasad (1983) observed increase in LDH activity of the gill and muscle of T. mossambica exposed to sublethal concentration of lindane. Sastry and Siddique (1983) reported elevated levels of LDH in the gill of C. punctatus exposed to endosulfan. Sreenivasa Murthy et al., (1983) reported increased LDH activity in the gill of L. marginalis treated with low concentrations of phosphamidon. Girija Moses (1984) reported increase in gill LDH activity of T. mossambica exposed to low concentrations of liver. Rangaswamy (1984) reported an increase in tissue LDH activity of T. mossambica exposed to multiple sublethal concentrations of endosulfan. Abidi (1986) observed an increase in LDH activity of the liver and muscle of fish on subacute exposure to endosulfan.
Rajandraprasad Naidu *et al.* (1986) observed a significant increase in LDH activity of the hepatopancreas, gill and claw muscle of *O. senex senex* exposed to sublethal and lethal concentrations of endosulfan. Azhar Baig (1988) reported enhanced LDH activity levels in the liver and muscle of *C. punctatus* exposed to sublethal concentrations of heptachlor. All the above authors have attributed an increase in LDH activity in hepatopancreas of *O. senex senex* by the occurrence of hypoxia under the treatment of sublethal concentrations of phosalone. Glycolysis is the pathway of the oxidation of glucose or glycogen to pyruvate and lactate. When oxygen is in short supply, reoxidation of NADH formed during glycolysis is impaired. Under these circumstances NADH in reoxidized by being coupled with the reduction of pyruvate to lactate, a reaction catalyzed by lactate dehydrogenase (LDH). The NAD so formed is used to allow further glycolysis to proceed. Glycolysis thus takes place under anaerobic conditions, but this limits the amount of energy liberate per molecule of glucose oxidized. Subsequently, to provide a given amount of energy, more glucose must undergo glycolysis under anaerobic as compared with aerobic conditions. These observations suggest that hypoxia (reduced availability of oxygen) would cause increase in LDH activity. Accordingly in the present study, the sublethal concentrations of phosalone altered markedly the whole animal oxygen consumption.
Enzymes Related to Energy Metabolism

and tissue oxygen uptake (Table 3.1) with a subsequent fall in energy production indicating the prevalence of anaerobiosis. Augmented anaerobiosis has been found to be associated with elevated LDH activity in animal tissues (Ranganatha Koundinya, 1978; Bhagyalakshmi, 1981; Sivaiah, 1981; Sivaprasada Rao, 1981; Rajeswari, 1989). Further phosalone is well implicated in producing hypoxia in different non-target organisms (Janardan Reddy, 1988; Narasimha Reddy, 1989). Diminished cellular oxidations leading to anaerobiosis could be one of the reasons for the elevation of LDH in the tissues. The LDH activity was estimated in the present study in the direction of pyruvate formation. The increase in LDH activity implies that the pyruvate production is not being affected during phosalone treatment. However, the pyruvate that is produced is not being properly utilized in the TCA cycle as evidenced by the inhibition of the activities of TCA cycle enzymes. Increased LDH and decreased SDH activity indicate prevalence of anerobic conditions in the tissues during phosalone exposure. Hence it may be concluded that hypoxia, induced by sublethal concentration of phosalone, could be responsible for an increase in LDH activity of the hepatopancreas of O. senex senex.

Glucose-6-phosphate dehydrogenase (G-6-PDH)

Table 3.3 presents the data on G-6-PDH activity of the hepatopancreas of O. senex senex exposed for 1, 3 and 7 days to sublethal concentrations of phosalone. The results show that there was a progressive increase in G-6-PDH activity and the percent increase for 1 day (13.84%), 3 day (30.76%) and 7 day (46.15%) observed being significant (P < 0.05). Several authors have studied the effects of different pesticides on tissue G-6-PDH activity of hepatopancreas and claw muscle of O. senex senex exposed to sublethal concentrations of sumithion. Satyaprasad (1983) reported an elevation in G-6-PDH activity of the liver, gill and muscle of T. mossambica subjected to lindane intoxication. Kalarani et al. (1984) recorded an increase in G-6-PDH activity of the hepatopancreas and foot muscle of P. globosa exposed to sublethal and lethal concentrations of endosulfan. Rangaswamy (1984) observed increase in G-6-PDH activity of the liver, gill and muscle and T. mossambica exposed to sublethal concentrations of endosulfan. Rajendraprasad Naidu et al. (1986) reported augmentation in G-6-PDH activity of the hepatopancreas, gill and claw muscle of O. senex senex exposed to sublethal and lethal concentrations of endosulfan. Rajeswari (1989) reported an increase in G-6-PDH activity of O. senex senex treated with
sublethal concentrations of endosulfan over a 30 day period. The above authors have attributed an increase in G-6-PDH activity to hypoxia resulting increase in energy metabolism as a compensatory mechanism.

Glucose-6-phosphate dehydrogenase a representative of the hexose monophosphate shunt pathway, catalyzes the dehydrogenation of glucose-6-phosphate to 6-phosphogluconolactone, a rate limiting reaction in the shunt pathway. It is known that under hypoxic conditions cellular oxidations are lowered resulting in reduced energy production. Under the stress of the energy demand and reduced energy output animals may seek to other means of energy production and HMP shunt is the important alternate pathway of providing energy since it accounts for the complete oxidation of glucose. Further HMP shunt is involved in providing precursors for the biosynthesis of nucleic acids, fatty acids and certain glucogenic amino acids and reduced NADP required by anabolic processes outside the mitochondria (Martin et al., 1983). Increased G-6-PDH activity in hepatopancreas indicates higher operation of the HMP pathway under phosalone exposure possibly to generate NADPH and pentose sugars. Since reduced fatty acid synthesis was observed during pesticide exposure (Bhatia and Venkatasubramaniyan, 1971), the NADPH required for the synthesis of fatty acids should be utilized for other purposes. Since, the NADPH also plays a vital role in the detoxification of pesticides (O’ Brien, 1967), it is likely that the NADPH generated through HMP pathway could in this case also be used for the detoxification of phosalone. Besides producing NADPH, G-6-PDH activity also contributes to the formation of pentose sugars which are needed for the synthesis of nucleic acids. This agrees with the earlier reports of Cappon Nicholls (1975) an increase in the nucleic acid content during pesticide treatment. Kabeer (1979) also reported an increase in the DNA and RNA contents of fish, T. mossambica species under malathion exposure. Since, the nucleic acids are associated with protein synthesis, increase in the nucleic acids should have increased the turnover of proteins observed in the present study (Chapter- IV, Table 4.4). Since liver is the site of nucleic acid synthesis and detoxification of pesticides, maximal increase in liver G-6-PDH activity under methyl parathion exposure (Sivaprasad Rao, 1980) may lead to production of pentoses and NADPH for the synthetic and detoxification purposes, possibly to mitigate pesticide toxic stress as an adaptive response. Contrary to the above results in recovery span ICDH, SDH and ATPase activities are progressively increased, where as LDH and G-6-PDH activities are
gradually decreased till day 7. On the 7th day of the recovery span the per cent difference between the controls and experimental values are statistically not significant (P < 0.05) (Tables 3.2 & 3.3).

In recovery span the activities of ICDH and SDH were progressively increased, indicating the probable recovery from the disruption of mitochondrial integrity and decrease of hypoxic condition in the crab. There is evidence of a recovery of whole animal oxygen consumption and tissue respiration in crab, after their shift from pesticide water to normal water (Table 3.1; Figure 3.1). The total ATPase activities in recovery span are also progressively increased resulting the recovery probably in permeability of mitochondrial membrane and an active transport phenomenon as well (Table 3.2; Figure 3.2).

The activity level of LDH showed a gradual recovery in crab in recovery span (Table 3.3) indicating shifting of metabolism from anaerobiosis to aerobiosis and increase the production of pyruvate to lactate. Hence then the liberation of energy is increased. The activity level of G-6-PDH showed a recovery trend from increase to decrease in recovery span of crab in normal water. In general, under stress condition HMP shunt is an alternative pathway to meet the energy demands and for detoxifying the pesticide by the production of NADPH. In recovery span the decreased G-6-PDH activity of the hepatopancreatic tissue indicating a lower operation of HMP pathway and decrease in the production of NADPH. The above results indicate that a gradual elimination of phosalone from the crab and then the animal might reach normal state.

It is also indicative of gradual elimination of the accumulated pesticides from the body and reshifting of energy metabolism from anaerobiosis to aerobiosis.