Lipid Metabolism
INTRODUCTION

Lipids or fats are heterogeneous group of complex molecules having high caloric value present in all biosystems. On complete oxidation 4 moles of glucose approximate 2744 kcal, whereas 1 ml of palmitate approximates 2400 kcal (Hoar and Randall, 1969). Essentially lipids are esters of fatty acids or substances capable of forming esters which consist of fats, oils, phospholipids, triglycerides, glycerol, cholesterol, neutral lipids etc. Lipids constitute not only the architecture of cells but also form co-basis for the structure of some enzymes like Mg$^{2+}$ ATPases. They offer full complementary structure to steroid hormones and also contribute for energy synthesis as an alternative to carbohydrates (Guyton, 1981; Sai chandra, 2002). Lipids are generally stored in the liver, adipose tissue and muscles, and are one of the most important fish energy sources. They are mobilized when food intake cannot supply the energy demands of growth and maintenance (Moreira et al., 2002).

The lipid source is contributed by the process of lipogenesis from carbohydrates in intermediary metabolism of fishes, that lipid accumulates extensively as an end product of carbohydrate metabolism. The incorporation of glucose carbon into lipid has been well reported in fishes, however, it varies between tissues (Hoar and Randall, 1969). The lipid requirement of fishes varies with the medium in which they are cultured (Benitez and Gorriceta, 1983).

Fish accumulate large lipid reserves prior to gonad development and most studies reveal that the lipid content of the viscera is inversely related to
the gonodosomatic index (Henderson and Tocher, 1987; Moreira et al., 2002). Utilization of lipid reserves involves several biochemical changes and the energy yielding processes. β-oxidation of fatty acids is known to occur after their release from triglycerides. The major characteristics of β-oxidation are 1) that the fatty acyl chain is shortened by two carbons at a time, 2) that it is the CoA derivatives and not the free fatty acids that are involved in these reactions, and 3) that the liberated two-carbon fragment is acetyl CoA, thus appear to be common to both fish and mammalian mitochondria (Hoar and Randall, 1969). Studies indicate that fish can use 20 to 35% of dry diet ingredients as fat, provided there is an adequate amount of choline, methionine and tocopherol present in the ration (Lee and Sinnhuber, 1972; Moreira et al., 2002). Utilization of fat in the diet improves the quality of flesh (Ellis and Reigh, 1991; Moreira et al., 2002) and also excess amount of lipid in the diet produces fatty fish (Cowey and Sangent, 1972).

The concentration of metabolic enzymes vary in fishes according to their size and growth rate (Pelletier et al., 1993), reproductive state (Kiessling et al., 1995), feeding status (Kiessling et al., 1989), physical injuries (Grizzle et al., 1992), and acclimation temperature (Seddon, 1997). Lipase is a metabolic enzyme associated with the reactions of lipolysis and lipogenesis i.e., breakdown of lipids into fatty acids and glycerol resulting in the release of water and energy; and the synthesis of neutral lipids by its reverse reaction, respectively. The physiological role of lipase in mobilization of lipids consists essentially in promoting the hydrolysis of fatty deposits (Bilinski, 1969) and the synthesis of neutral fats by its reverse action. Earlier studies on lipase activity
were directed towards correlating its activity to the general activity of the fish. In general, the lipase activity is higher in more active fish than in those forms which are sluggish. Fish muscle is known to contain a lipase capable of catalyzing the hydrolysis of short – chain triglycerides rather than the long – chain ones (George, 1962).

Life is said to have orginated in an aqueous medium and the various chemical reactions involved in metabolism to sustain life take place in aqueous environment. Nevertheless life is possible only because of the existence of hydrophobic molecules which by being squeezed out of aqueous solutions and stacked together in well defined patterns constitute barriers limiting most metabolic reactions to specified areas. Such molecules are called amphipathic lipids (i.e., those having a polar hydrophilic moiety and a polar hydrophobic end), the phospholipids and glycolipids come under this category. The site of phospholipid biosynthesis is believed to be endoplasmic reticulum and not plasma membrane or golgiapparatus (Singh and Shankar, 1996).

Glycerol is one of the products of fat hydrolysis, part of which is utilized for metabolic energy production and the rest of it is for phospholipid synthesis (Lin, 1977). The other product of fat hydrolysis is free fattyacids. Through β-oxidation from acetyl CoA, they are partly channeled into the TCA cycle for metabolic energy production and rest to the production of acetoacetate. This acetoacetate, together with that produced from other sources may have been partly oxidized to metabolic energy production and the rest is utilized for the synthesis of cholesterol, as the precursor. In general fattyacids are mobilized
from the neutral lipid reserves of fish adipose tissue during gonadogenesis and transferred via the serum to the liver where they are assembled into the lipoprotein and vitellogenin (Moreira et al., 2002).

Vertebrates are known to biosynthesize cholesterol from precursors such as acetate and mavalonate (Chandge and Paul Raj, 1997). Cholesterol, in turn, serves as the precursor for steroid hormone synthesis (Aditya kumar, 1980). Huhn and Robinson (1966) reported increasing plasma cholesterol levels with increasing activity of the fish. However, this is contradicted by Larsson and Fange (1977) on the basis of the findings that while highly inactive fish like Cyclopterus lumpus had high levels of plasma cholesterol, very active fish like Gades virens and Clupea herengus had low or only intermediate levels of plasma cholesterol. The role of cholesterol seems to be considerably important. Cholesterol may inhibit the activity of Na⁺ - K⁺ ATPase by reducing the ability of protein to penetrate the bilayer. Together with the fatty acyl chain length and lipid unsaturation, cholesterol may also be concerned with the control of the fluidity of the membrane lipids (Kimelberg and Papahadjopoulos, 1974). Cholesterol occurs in plasma membranes of many animal cells in the lipoprotein of plasma and large quantities occur in the brain and nerve tissue (Chandge and Paul Raj, 1997).

Biochemical changes induced in the different tissues of fishes by pollutants have been studied in significant details, but such studies on fat metabolism are limited. To mitigate any stress condition for the fish, generally, more energy is needed, which may be obtained from carbohydrates, proteins, and / or lipids. Involvement of lipid metabolism in fishes exposed to toxic
stress can be expected based on two grounds. The first is to provide necessary energy to meet additional energy demands of various active processes involved during toxic stress. The second one is to provide at least a part of the extra amount of water needed for the regulation of osmoconcentration of body fluids. Lipid and water contents have been reported to be inversely proportional to each other in fishes (Ganesan et al., 1989).

Siva Prasada Rao and Ramana Rao (1981) reported a decline in total lipids and phospholipids, and an elevation in free fatty acids and total cholesterol in the red muscle, gill, liver and brain tissues of *Tilapia mossambica* exposed to the sublethal concentration of methyl parathion. The same species showed increased levels of total lipids in kidney under sublethal heptachlor intoxication (Radhalah et al., 1987). Jaishree Pant et al., (1987) studied the chronic effect of aldicarb on the blood and tissues of *Barbus conchronius* and reported hypercholesterolemia in its blood, liver and ovary; elevation in liver lipid and free fatty acid levels; and a decrease in muscle lipids, however, the liver showed a profound increase. *Oreochromis mossambicus* showed a general decline in the lipid content of liver tissue on exposure to the sublethal concentration of endosulfan (Ganesan et al., 1989). Anupam Jyothi et al., (1989) reported a decrease in phospholipids in liver, heart and dorsal muscle of *Channa punctatus* during acute intoxication of malethion. The same fish showed significant depletion of lipid content in liver, brain, testis and ovary; and a significant elevation of cholesterol as a result of chronic effect of nuvian (Ghosh and Chatterjee, 1989). This fish on exposure to sublethal concentrations of vegetable oil factory effluent showed a decrease in total lipid content in liver (Saroj Gupta, 1987). Singh, (1992)
observed a decrease in total lipid content in the liver of *Labeo rohita* exposed to the sublethal concentration of malathion. Saichandra (2002) reported a decrease in total lipid content, and an increase in free fatty acid levels and the activity of lipase in gill, liver and muscle of *Labeo rohita* exposed to the sublethal concentration of ziram.

The reports on the lipid metabolism of freshwater fishes exposed to heavy metals, however, are very limited. Fatty infiltration of liver is very common in the cases of fishes exposed to chemical stressors, particularly the heavy metals and xenobiotics (Couch, 1975). Hema Tewari et al., (1987) studied the impact of chronic lead poisoning on the haematological and biochemical profiles of *Barbus conchronius* and reported decreased levels of cholesterol in its blood, liver, ovary and testis. Gokhale and Patil (1989) studied the effect of four heavy metals Cd, Pb, Cu and Zn on different organs of *Mystus quilo* under fluctuating environmental factors, seasonally, and observed that the protein and lipid contents remained as it has developed tolerance inspite of its high position in tropic webs. Virk and Sharma (2003) observed the changes in the biochemical constituents of chromium exposed *Cirrhinus mrigala* and observed upsurge in lipids levels in its muscle. Vutukuru (2003) studied the chromium induced alterations in some biochemical profiles of Indian major carp *Labeo rohita* and reported a decrease in total lipid content in its gill, liver and muscle.

Information regarding the effect of lead and chromium on lipid metabolism of freshwater fishes is scanty. Possible changes in lipid metabolism were reported on the basis of its significant role in the
physiological processes of the fish during lead and chromium stress (Rana and Sharma, 1982; Verma et al., 1986). Most of these studies, however, used acute exposures of lead and chromium and the agreement between the actual and nominal concentrations was not reported. No literature, however, is available on the energetics based on the involvement of lipid profiles in freshwater fish in relation to the stress caused by the lead and chromium.

Based on the above assumptions it is of present interest to know how the common carp could respond to the stress caused by the sublethal concentrations of lead and chromium by involving in its lipid metabolism. So an attempt is made in this study to observe the effect of sublethal concentrations of lead and chromium on certain aspects of lipid metabolism like total lipids, free fatty acids, lipase activity, phospholipids, and cholesterol in the gills, brain, liver and muscle of the fingerlings of C. carpio on short – term and long – term exposures.

RESULTS

The data on the levels of total lipids, free fatty acids, phospholipids and cholesterol (mg/g wet wt) and the activity of lipase (lipase units/g wet wt) in gills, brain, liver and muscle of C. carpio at 1, 7, 15 and 30 days on exposure to the sublethal concentrations of lead and chromium, besides controls, are presented in tables 28 to 32. For comparison, the differences obtained in the levels of total lipids, free fatty acids, phospholipids, cholesterol and lipase activity in each organ of the fingerlings between the controls and the respective exposure periods in the sublethal concentration of lead and chromium were converted as percentages of the corresponding control level /
activity and these values are also given in the same tables and plotted against exposure periods in figures 26 to 30.

**Total lipids as a function of lead and chromium toxicity**

Relative to controls the level of total lipids recorded a decrease in gills, brain, liver and muscle of fingerlings subjected to the sublethal concentration of lead. The decrease in total lipids was significant (P<0.05) in almost all organs of the fingerlings at all the exposure periods, except in the brain at day 1. In the sublethal concentration of chromium the organs of fingerlings showed a gradual increase in total lipid level at all the exposure periods and this increase was mostly insignificant (P>0.05) at both 1 and 7 days. The increase observed in total lipid level at 1 and 7 days in the organs of fingerlings exposed to the sublethal concentration of chromium was very less.

Among the exposure periods the decrease observed in the levels of total lipids in the organs of fingerlings exposed to the sublethal concentration of lead differed in degree. It was more pronounced at 30 days and less at 1 day in the order 1<7<15<30 days. The differences between 1 and 30 days were statistically significant (P<0.05). In the organs of fingerlings exposed to the sublethal concentration of chromium the increase in lipid level though greater at 7 days than at 1 day, the differences in them were not significant (P>0.05). But the increase was greater at 30 days than at 15 days with significant (P<0.05) differences between these two exposure periods (table 28; figure 26).

Among the organs studied the magnitude of decrease in total lipid levels in the organs of fingerlings exposed to the sublethal concentration of
lead was in the order gills > liver > muscle > brain. However, the differences between liver and gills, brain and muscle were mostly insignificant (P>0.05). In the sublethal concentration of chromium the increase in total lipid levels was greater in degree in the muscle less in brain in the order muscle > gill > liver > brain. However, the differences among the organs were mostly insignificant (P>0.05).

**Free fattyacids as a function of lead and chromium toxicity**

Relative to controls, the levels of free fattyacids recorded an increase in gills, brain, liver and muscle of fingerlings subjected to the sublethal concentration of lead. The increase in free fattyacids level was significant (P<0.05) in almost all organs of fingerlings at all the exposure periods studied, except in muscle at day 1 where an insignificant (P > 0.05) increase was observed. The fish exposed to the sublethal concentration of chromium showed a decrease in free fattyacid levels at all the exposure periods and this decrease was mostly significant (P<0.05) at all days of exposure, except at day 1 and in muscle at day 7. Overall the changes in free fatty acids were significant at 30 day of exposure than at day 1, 7 and 15 in sublethal concentrations of lead and chromium (table 29; figure 27).

Among the exposure periods the increase observed in the free fattyacids levels in the organs of fingerlings exposed to the sublethal concentration of lead differed in degree. It was more at 30 days and less at 1 day in the order 1<7<15<30 days. The differences between 1 and 30 days were statistically significant (P < 0.05). In the organs of fingerlings exposed to the sublethal concentration of chromium the degree of decrease in free
fatty acid levels was more at 30 days than at 1, 7 and 15 days and the differences in between the any two exposure periods were significant (P<0.05).

Among the organs studied the magnitude of increase in free fatty acid levels in the organs of fingerlings was in the order: gills > liver > muscle > brain. And the differences between liver and gills, and brain and muscle of fingerlings were mostly significant (P < 0.05). In the sublethal concentration of chromium the degree of decrease in free fatty acid levels was greater in the brain and less in liver in the order: brain > muscle > gills > liver. And, the differences among the organs were mostly significant (P < 0.05) (table 29; figure 27).

**Lipase activity as a function of lead and chromium toxicity**

Corresponding to the changes in total lipids and free fatty acids the activity of lipase recorded an increase relative to controls in the gills, brain, liver and muscle of fingerlings subjected to sublethal concentration of lead. The increase observed in lipase activity was mostly significant (P<0.05) at all the exposure periods studied. In sublethal concentration of chromium the lipase activity showed a significant decrease in all the organs of fingerlings at all the exposure periods except at day 1 in gills, brain and muscle and at day 7 in liver. However, this decrease was insignificant (P> 0.05) at those days of exposure (table 30; figure 28).

Among the exposure periods the degree of increase in lipase activity in all the organs of fingerlings was less at 1 day and more at 30 days on
exposure to the sublethal concentration of lead, and it was in the order 1<7<15<30 days. In the sublethal concentration of chromium the decrease in the lipase activity followed the above order in all the organs of fingerlings.

Among the organs, in lead exposed animals, the increase in lipase activity was in the order: gills > liver > brain > muscle, with significant differences between gills and muscle, and brain and liver. Whereas in the fish exposed to the sublethal concentration of chromium the decrease in lipase activity was in the order muscle > brain > gills > liver and these differences were mostly significant.

**Phospholipids as a function of lead and chromium toxicity**

Similar to the changes in total lipids, the levels of phospholipids recorded a decrease in gills, brain, liver and muscle of fingerlings of *Cyprinus carpio*, relative to controls, on exposure to the sublethal concentration of lead. The decrease observed was mostly significant (P<0.05) at all the exposure periods studied. However, it was more pronounced and significant at day 30 than at days 1, 7 and 15, thus the decrease increased over time of exposure (day 1<7<15<30). In sublethal concentration of chromium the levels of phospholipids increased in gills, brain, liver and muscle of fingerlings of *Cyprinus carpio* and this increase was mostly significant at all the exposure periods, except at day 1. The increase progressed over time of exposure (day 1<7<15<30) (table 31; figure 29).

Among the exposure periods, the degree of decrease in the levels of phospholipids in all the organs of fingerlings was less at 1 day and more at 30
days on exposure to the sublethal concentrations of lead, in the order 1<7<15<30 days. In sublethal concentration of chromium the phospholipid levels increased in all the organs of fingerlings with the increase in exposure period, and it was in the order: 1<7<15<30 days.

Among the organs studied at any exposure period in the sublethal concentration of lead the decrease in phospholipids was in general greater in magnitude in muscle than in remaining organs of fingerlings. Thus the degree of decrease in phospholipids, followed the order muscle > gills > liver > brain. But, whereas in sublethal concentration of chromium the increase in phospholipids in the organs of fingerlings of *C. carpio* followed the order muscle > gills > brain > liver.

**Cholesterol as a function of lead and chromium toxicity**

Relative to controls the level of cholesterol increased in gills, brain, liver and muscle of fingerlings of *Cyprinus carpio* exposed to the sublethal concentration of lead at all the exposure periods studied. The increase was mostly significant (P<0.05) in all the organs of fingerlings except in the muscle at day 1. Further, the increase was significantly greater at 30 days than at day 1, 7 and 15. In sublethal concentration of chromium also the cholesterol level increased in all the organs of fingerlings of *Cyprius carpio* at all the exposure periods, but this increase was relatively less compared to the increase in sublethal concentration of lead. Further, the increase decreased overtime of exposure (day 1 > 7 < 15 < 30) and it was not significant in the brain at day 30, and in muscle at day 7 and 30 (table 32; figure 30).
Among the exposure periods, the increase in cholesterol level in all the organs of fingerlings was less at 1 day and greater at 30 days on exposure to the sublethal concentration of lead with the order: 1 < 7 < 15 < 30 days. In sublethal concentration of chromium the increase in cholesterol level progressively decreased in all the organs of fingerlings in the order: 1 > 7 > 15 > 30 days.

Among the organs the degree of increase in cholesterol level was in the order: gills > liver > brain > muscle in sublethal concentration of lead at all the exposures studied. Whereas in chromium exposed fingerlings the degree of increase was in the order: liver > gills > brain > muscle (table 32; figure 30).

DISCUSSION

Involvement of lipids in fishes exposed to toxic stress can reasonably be expected on two grounds: one is to meet additional energy demands and the other is to provide apart an extra amount of water needed for osmo-concentration of body fluids. Studies on the involvement of lipids in freshwater fishes exposed to heavy metals are limited (Urmila Devi and Radhakrishnaiah, 1990; Radhakrishnaiah et al., 1991b; Sivaramakrishna et al., 1992). These studies reported both increase and decrease of lipids correlated to the concentration of metal. But, no studies are available on the effect of lead and chromium on lipids exposed to the sublethal concentrations. In the present study the decrease in total lipids in the gills, brain, liver and muscle of fingerlings of C. carpio exposed to the sublethal concentration of lead at the exposure periods, 1, 7, 15 and 30 indicates the breakdown of these biomolecules due to subacute toxicity stress of the metal. However, the
degree of breakdown is dependent on the organ of the fish and length of exposure period. The decrease in the total lipid content might be due to the utilization of it to meet the energy demand associated with the situation of stress (Rao and Rao, 1981; Ganesan et al., 1989; Sai Chandra, 2002).

In fishes free fatty acids have been well studied in relation to starvation (Goren Dave et al., 1975; Goren Dave, 1977; Gupta et al., 1994). These studies indicated increased utilization of free fatty acids during stress conditions. It is possible that free fatty acids may undergo β-oxidation leading to the formation of acetyl CoA which enters into TCA cycle for energy release. Apart of this, acetyl CoA may be used for the synthesis of cholesterol or for the synthesis for acetoacetate in the liver. This acetoacetate is, however, transported to the extra – hepatic organs where it is subjected to oxidation leading to the production of energy. In the present study the increase in free fatty acid levels corresponding to the decrease in total lipids in almost all the organs of fingerlings exposed to the sublethal concentration of lead at all the exposure periods studied indicates the mobilization of lipids to meet the energy demands (Gupta et al., 1994; Saxena and Gupta, 1997; Kumar, 1999; Prabhu and Kumar, 2001). The oxidation of free fatty acids thus liberated, however, might be greater at day 1 of exposure as the increase in free fatty acids was insignificant relative to the decrease in total lipids. But more accumulation of free fatty acids at day 30 of exposure could be due to the decreased availability of oxygen, thereby the rapidity of β-oxidation might have decreased leading to the increase in free fatty acid levels. However, part of the free fatty acids formed might be converted into ketone bodies by enhancing the ketogenesis in order to liberate energy to meet the demands.
Lipase activity is known to present in organs of fishes (Patton et al., 1975; Urmila Devi, 1993), however, there is little information on its role during toxic stress. Increase in lipase activity in the organs of fingerlings on exposure to the sublethal concentration of lead could be taken to indicate the lipid breakdown, the lipolysis. This is clearly evident by the decrease in total lipids and increase in the levels of free fatty acids. The induction of severe breakdown of lipids could cause the disintegration in the structural organisation of the fish, which in turn could lead to the imbalance in its homeostatic mechanism and the failure of metabolic compensation and regulation (Sivaramakrishna et al., 1992). As the animals are continuously exposed to sublethal concentration of lead for a long period of study the intensity of lipolysis increased over time of exposure i.e., from day 1 to day 30. Similar changes in lipase activity were also observed in fishes over a length of exposure to toxicants, especially pesticides (Rath and Misra, 1980; Manoharan and Subbaiah, 1982; Ganesan et al., 1989; Urmila Devi, 1993).

The phospholipids also decreased corresponding to the decrease of total lipids and to the increase of lipase activity and free fatty acids in the organs of fingerlings exposed to sublethal concentrations of lead at all the exposure periods. The degradation of phospholipids facilitates the turn over of triglycerides (Harper et al., 1979). A correlation had been suggested between phospholipids and lipolytic activity (Prosdocimi et al., 1977) and it was pointed out that phospholipids enhance lipolytic activity. Since phospholipids are essential for the activity of Na\(^+\) - K\(^+\) ATPase (Knowles et al., 1975; Vutukuru, 2003) their decrease results in the inhibition of this enzyme activity, which is also evident in the present study. The decrease in the level of phospholipids
in the organs of the fish may be due to oxidative degradation of membranes (Anupam Jyothi et al., 1989).

Cholesterol in contrast to total lipids and phospholipids and parallel to the lipase activity and free fatty acids increased in the organs of fingerlings on exposure to sublethal concentration of lead at all the exposure periods studied i.e., day 1, 7, 15 and 30. As mentioned already, the β-oxidation results in the production of acetyl CoA. The increase in the total cholesterol content in the organs suggests increased diversion of high amounts of acetyl CoA produced from the β-oxidation to acetoacetate for the synthesis of cholesterol. This diversion may be expected as there is possibility of accumulation of acetyl CoA, since Kreb’s cycle enzymes could be inhibited during stress conditions (Kabeer et al., 1978). Besides as toxicants inhibit the metabolism of steriods (Kupfer, 1969), the cholesterol increase might also be due to its non-utilization for the synthesis of steroidal hormones. The impaired steroidogenesis could cause the accumulation of cholesterol in tissues (Singh and Singh, 1980). Cholesterol is known to control the fluidity of the membrane lipids which in its turn may control the activity of the membrane bound enzymes like Na⁺ - K⁺ ATP ases (Kimelberg and Papahadjopoules, 1974). Further, cholesterol is known to lower the membrane permeability (Aditya Kumar, 1980). These findings suggest that metabolic regulation under the influence of cholesterol is effected in the fingerlings of C. carpio through lowering of the transport of metabolites, and perhaps also ions into the cell in order to counteract the lead toxicity. Thus cholesterol inhibits the activity of the membrane bound enzymes like Na⁺, K⁺ – ATP ases (Parvateswara Rao, 1970; Scheer and Langford, 1976).
Gradual increase in total lipids and phospholipids along with the corresponding decrease in lipase activity, free fatty acids and cholesterol in almost all the organs of fingerlings exposed to the sublethal concentration of chromium indicate the operation of lipogenesis. Increase in lipids could be to maintain the dynamic metabolic functions of the organs of the fish in order to resist the sublethal toxic stress. The decrease in cholesterol could be due to the partial diversion of it to lipogenesis. In addition to it the water content may also decreased in favour of increased total lipids. Since the perturbance caused to the fish by the sublethal chromium stress not as much severe as seen in the case of sublethal lead stress, probably the fish need not go for lipids to derive energy; the reserve carbohydrates might be sufficient to meet the required demands. Further, to strengthen the structural integrity the synthesis of phospholipids enhanced (Lin, 1977). Since phospholipids are essential for the activity of Na⁺ - K⁺ ATPase (Bruni et al., 1974; Knowles et al., 1975; Vutukuru, 2003) their increase facilitates the greater activity of this enzyme system. The increased activity is evident in the present study in the organs of fingerlings exposed to the sublethal concentration of chromium.

Further, the decrease in lipase activity with the increase of total lipids and decrease in fatty acids, lower the rate of β-oxidation thereby the release of acetyl – CoA also decreases which in turn decreases the cholesterol synthesis resulting the decreased levels of cholesterol. The cholesterol though produced in small amounts would be utilized for the steroid hormonal synthesis as the precursor, hence there exhibited a decrease especially in all the organs of fish exposed to the sublethal concentration of chromium. Swami et al., (1983) observed a metabolic shift from carbohydrate to lipid metabolism.
through Acetyl – CoA barrier leading to an increment in lipids in the organs of freshwater mussel, *Lamellidens marginalis*, under pesticide toxicity. Perhaps, the same reason may hold good for the increased lipogenesis in the organs of the fish under sublethal chromium stress. The lipoproteins and phospholipids may constitute the structural rigidity to prevent the entry of toxic ions. This could not be possible in sublethal concentration of lead, because the bound lead has some finite limit with regard to intracellular storage, as reported in sublethal concentration of mercury (Urmila Devi and Radhakrishnaiah, 1990).

The differences observed in the degree of decrease in the total lipids and phospholipids among the organs of the fingerlings exposed to the sublethal concentrations of lead can be attributed to the concentration of metal accumulated in them, as it is also conjunctured that cells have evolved their own methods of solubilizing, transporting, regulating, detoxifying and excreting these metals (Gokhale and Patil, 1989). The high lipolytic activity in the gills of fish indicates the disruption of gill lamellae and dissolution of cellular fragments, which is also evident by its histological structure. The greater increase in the free fattyacids and cholesterol in the gills could be due to their mobilization from the major site of the synthesis, liver, through blood, as gills are more vascularised structures compared to the other organs (Siva Prasada Rao and Ramana Rao, 1981). In sublethal concentration of chromium, the increase in total lipids and phospholipids together with the decrease in free fattyacids and cholesterol could aid fortification of their structural rigidity to perform both osmo-and Ino-regulatory and respiratory functions (Siva Ramakrishna *et al.*, 1992), as the Na\(^+\) - K\(^+\) and Mg\(^{2+}\) ATP ases are the lipid bound enzymes (Renfro *et al.*, 1974).
Besides generally being an important centre for metabolism including interconversion and storage of food stuffs, liver is an important centre for lipid storage and is known to play a key role in the synthesis and oxidation of fatty acids and in the synthesis of phospholipids and cholesterol. Further, liver is known to possess the enzymes for ketogenesis involving the conversion of fatty acids to ketonebodies and to produce, under certain metabolic conditions, considerable amounts of acetoacetate which is transported via the circulating blood to various extra hepatic tissues where it is utilized for metabolic energy production (Harper, 1977). In view of these facts it appears that many functions of liver are involved in the fish to meet the toxic stress. High concentrations of metals in liver with no apparent relationship with their levels in water considerably affect its cellular integrity (Gokhale and Patil, 1989). A decrease in total lipids was reported in fish liver during toxic stress (Ghosh and Chatterjee, 1989; Ganesan et al., 1989; Anupan Jyothi et al., 1989; Sai Chandra, 2002). Rao and Rao (1981) reported a decrease in phospholipids in fish liver during methyl paration intoxication. Similarly in the present study in the fingerlings liver the total lipids and phospholipids decreased on exposure to sublethal concentration of lead. The depletion in liver lipids could be either due to active uptake of lipid components by the other tissues for utilization or increased lipolysis or mitochondrial injury which impair the function of citric acid cycle and fattyacid oxidation mechanism (Singh and Singh, 1980; Ware, 1980). The increase in lipase activity and free fatty acids is an indication of the increased rates of lipid lipolysis. Jaishree Pant et al., (1987) reported an increase in the fish liver fattyacids during chronic intoxication of different pesticides. Fatty infiltration of liver is very
common in case of exposure to chemical stressors, particularly the heavy metals and xenobiotics (Couch, 1975). This gives support to the elevation in free fatty acids in the liver of fingerlings in the present study. Some reports are available on hypercholesterolemia in fish liver during pesticide intoxication (Ghosh and Chatterjee, 1989; Anupam Jyothi et al., 1989). Liver cholesterol showed marked increase in fish tissues due to the breakdown/damage of structural parts of hepatocytes (Deodhat and Bapat, 1984). Thus liver appears to be the target of deleterious effects of toxicants (Jaishree Pant et al., 1987) and it occupies a pivotal position in the metabolism of cholesterol as it does in the case of other lipids.

Liver is the main center for synthesis, transport and storage of lipids and is known to play a key role in the synthesis and inter conversion and storage of food stuffs (Plaa, 1992; Prabhu and Kumar, 2001). In contrast to the exposure of fish to sublethal concentration of lead, the increase in total lipids and phospholipids with a decrease in lipase activity, free fatty acids and cholesterol in the liver of fingerlings exposed to sublethal concentration of chromium might be for the synthesis of useful derivatives that help to mitigate to sublethal toxic stress. The increase in liver lipid is reported during the aldicarb sublethal exposure (Jaishree Pant et al., 1987). Sastry and Gupta (1979) observed localization of a considerable amount of lipid in the veins and its deposition in the neighbouring areas of liver of sublethal cadmium exposed Channa punctatus. Hypcholesterolemia in the fish liver on the impact of chronic lead poisoning has been reported (Hema Tewari et al., 1987). Depressed cholesterol levels may be related to its enhanced utilization in cortico-steroidogenesis. Involvement of thyroid hormones has also been
suggested in cholesterol metabolism and an enhanced breakdown in hyperthyroidism is known to result in hypocholesterolemia (Wasserman et al., 1970). In the bluegills, *Lepomis macrochiru*, exposure to methyl mercuric chloride caused a decrease in cholesterol (Dutta and Haghighi, 1986). Increased levels of serum high density lipoproteins was suggested to be the cause of hypocholesterolemia. The same reason might be attributed to the decreased levels of cholesterol in the fingerlings liver exposed to the sublethal concentration of chromium in the present study.

Muscle being essentially a contractile effect of organ with predominant participation in locomotion the decrease in total lipids and phospholipids and with the moderate increase in free fatty acids, lipase activity and cholesterol in this organ of the fingerlings exposed to the sublethal concentration of lead may indicate the utilization of lipids for energy production in meeting the synaptic transmittory activity at the myoneural junctions and ionic fluxes in the myofibrils. In this connection it is to note that the erratic swimming, hyperexcitability and increased irritability were observed in the fish exposed to sublethal concentration of lead, part of the energy for all these activities might have obtained by the lipid utilization. The increase in total lipid content observed in the muscle of fingerlings exposed to sublethal concentration of chromium may constitute mostly glycolipids, lipoproteins and phospholipids to form the structural entity to meet the sublethal chromium toxic stress under favourable conditions. The decrease in lipase activity may be to activate the lipogenesis in this effector organ. Involvement of the fish muscle lipid content during stress is well known. Rao and Rao (1981) and Jaishree Pant et al., (1987) reported a decrease in total lipid and phospholipid contents in the
An upregulated phospholipid content might be due to oxidative degradation in membranes indicating that the pectoral muscles have more demand for energy, and that the increase in ventral side muscles might be due to hypertrophy. In view of the evidence that cholesterol inhibits the activity of the membrane-bound enzymes (Scheer and Langford, 1976), it may be presumed that the increased cholesterol content might be the reason for decreased ionic fluxes in myofibrils.

The central nervous system plays an important role in the integration of various physiological processes of the animal (Lagerspetz, 1974). The mitochondrial components of the brain possess considerable redox potentials (Cutkomp et al., 1984). Even though the magnitude of changes observed in the lipid metabolism of brain in fingerlings exposed to sublethal concentrations of lead and chromium is less, the trend is almost similar to that of other organs. Since lead is known to be neurotoxic, the decrease in total lipids in this organ of the fish exposed to sublethal concentration may be mostly due to the breakdown of unsaturated triglycerides into fatty acids and glycerol. This alters the membrane permeability and affects efficient functioning of nervous system. The increase in total lipids and phospholipids in sublethal concentration of chromium could be for the effective functioning of the membrane permeability and nerve transmission. A decrease in total lipids and phospholipids and an increase in free fatty acids and cholesterol were noticed.
in the fish brain during sublethal toxic stress (Ghosh and Chaterjee, 1989). The lipid reserve of brain is meant for emergency purposes as reported by Narasimhan and Sundararajan (1971), hence in the present study the total lipids and phospholipids of fingerlings brain were least affected, especially in sublethal concentration of chromium.

In the present study the greater decrease in the total lipids, phospholipids and the increase in lipase activity, free fattyacids and cholesterol level in the gills, brain, liver and muscle of fish on exposure to sublethal concentration of lead could be due to the higher rate of accumulation of metal ions. Probably the intensity of lypolysis is dependent on the rate of accumulation. In lead exposed fish, increased rate of accumulation of metal over time of exposure might have severely affected the organs of them. Further more the internal defensive mechanisms and the immunochemical processes might have failed to dominate the metal effect. Therefore the degree of lypolysis is greater in the organs of lead exposed fish. But in chromium exposed fish, the greater activation of lipid synthetic potentials were observed, than the lead exposed ones. It is possible if the concentration of chromium accumulated within the organs of fish is in the limits of detoxification. For the activation of such detoxification mechanisms, lipids, probably, are more useful for structural rigidity to prevent the entry of toxic ions. The decrease in cholesterol level, in the organs of chromium exposed fish, than the fish exposed to lead could be due to the partial diversion of it to lipogenesis. The overall shifts in lipid metabolism, however, indicate that the sublethal concentration of lead is adversely effective to the fish, but they can resist to the sublethal concentration of chromium.
TABLE - 28
Total lipids (mg/g wet wt) in the organs of *C. carpio* at different periods of exposure to sublethal concentration of lead and chromium. Each value is a mean of six replicants. Percent change over the respective control is given in parenthesis.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Control</th>
<th>Lead</th>
<th>Exposure period in days</th>
<th>Chromium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Gill</td>
<td>41.28</td>
<td>37.20</td>
<td>31.25</td>
<td>29.90</td>
</tr>
<tr>
<td>S.D±</td>
<td>1.23</td>
<td>0.98</td>
<td>0.62</td>
<td>0.50</td>
</tr>
<tr>
<td>%</td>
<td>(-9.88)</td>
<td>(-24.29)</td>
<td>(-27.56)</td>
<td>(-49.49)</td>
</tr>
<tr>
<td>Brain</td>
<td>83.84</td>
<td>80.18*</td>
<td>76.15</td>
<td>69.54</td>
</tr>
<tr>
<td>S.D±</td>
<td>2.59</td>
<td>2.04</td>
<td>1.89</td>
<td>1.32</td>
</tr>
<tr>
<td>%</td>
<td>(-4.36)</td>
<td>(-9.17)</td>
<td>(-17.05)</td>
<td>(-25.27)</td>
</tr>
<tr>
<td>Liver</td>
<td>75.48</td>
<td>63.11</td>
<td>57.44</td>
<td>42.25</td>
</tr>
<tr>
<td>S.D±</td>
<td>1.45</td>
<td>0.86</td>
<td>0.64</td>
<td>0.50</td>
</tr>
<tr>
<td>%</td>
<td>(-16.38)</td>
<td>(-23.90)</td>
<td>(-44.02)</td>
<td>(-49.13)</td>
</tr>
<tr>
<td>Muscle</td>
<td>27.69</td>
<td>25.50*</td>
<td>22.12</td>
<td>20.19</td>
</tr>
<tr>
<td>S.D±</td>
<td>0.49</td>
<td>0.40</td>
<td>0.39</td>
<td>0.35</td>
</tr>
<tr>
<td>%</td>
<td>(-7.90)</td>
<td>(-20.11)</td>
<td>(-27.08)</td>
<td>(-29.10)</td>
</tr>
</tbody>
</table>

The differences between control and experimental are statistically significant (P<0.05).
* Denotes not significant with control (P>0.05).
TABLE 29
Free fatty acids (mg/g wet wt) in the organs of *C. carpio* at different periods of exposure to sublethal concentration of lead and chromium. Each value is a mean of six replicants. Percent change over the respective control is given in parenthesis.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Control</th>
<th>Exposure period in days</th>
<th>Lead</th>
<th>Exposure period in days</th>
<th>Chromium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gill</td>
<td>5.25</td>
<td>28.26</td>
<td>25.60</td>
<td>22.19</td>
<td>19.11</td>
</tr>
<tr>
<td>S.D±</td>
<td>1.26</td>
<td>0.99</td>
<td>0.92</td>
<td>0.60</td>
<td>0.53</td>
</tr>
<tr>
<td>%</td>
<td>(+438.28)</td>
<td>(+387.61)</td>
<td>(+322.66)</td>
<td>(+264.00)</td>
<td>(-0.95)</td>
</tr>
<tr>
<td>Brain</td>
<td>10.96</td>
<td>14.23</td>
<td>17.21</td>
<td>19.18</td>
<td>20.08</td>
</tr>
<tr>
<td>S.D±</td>
<td>0.72</td>
<td>0.85</td>
<td>0.89</td>
<td>1.03</td>
<td>0.49</td>
</tr>
<tr>
<td>%</td>
<td>(+29.83)</td>
<td>(+57.02)</td>
<td>(+75.00)</td>
<td>(+83.21)</td>
<td>(-4.19)</td>
</tr>
<tr>
<td>Liver</td>
<td>15.50</td>
<td>40.23</td>
<td>42.18</td>
<td>41.21</td>
<td>40.19</td>
</tr>
<tr>
<td>S.D±</td>
<td>2.20</td>
<td>1.99</td>
<td>1.70</td>
<td>2.00</td>
<td>0.86</td>
</tr>
<tr>
<td>%</td>
<td>(+159.54)</td>
<td>(+172.12)</td>
<td>(+165.87)</td>
<td>(+159.29)</td>
<td>(-2.12)</td>
</tr>
<tr>
<td>Muscle</td>
<td>7.18</td>
<td>7.39*</td>
<td>9.21</td>
<td>10.22</td>
<td>13.18</td>
</tr>
<tr>
<td>S.D±</td>
<td>0.39</td>
<td>0.42</td>
<td>0.56</td>
<td>0.54</td>
<td>0.40</td>
</tr>
<tr>
<td>%</td>
<td>(+2.92)</td>
<td>(+28.27)</td>
<td>(+42.33)</td>
<td>(+83.56)</td>
<td>(-1.39)</td>
</tr>
</tbody>
</table>

The differences between control and experimental are statistically significant (*P*<0.05).

* Denotes not significant with control (*P* > 0.05).
**TABLE - 30**

Lipase activity (Lipase units/g wet wt) in the organs of *C. carpio* at different periods of exposure to sublethal concentration of lead and chromium. Each value is a mean of six replicants. Percent change over the respective control is given in parenthesis.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Control</th>
<th>Lead</th>
<th>Exposure period in days</th>
<th>Chromium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>7</td>
<td>15</td>
</tr>
<tr>
<td>Gill</td>
<td>145.2</td>
<td>173.5</td>
<td>200.2</td>
<td>211.2</td>
</tr>
<tr>
<td>S.D±</td>
<td>5.32</td>
<td>6.30</td>
<td>5.09</td>
<td>5.81</td>
</tr>
<tr>
<td>%</td>
<td>(+19.49)</td>
<td>(+37.87)</td>
<td>(+45.45)</td>
<td>(+119.14)</td>
</tr>
<tr>
<td>Brain</td>
<td>133.80</td>
<td>184.70</td>
<td>190.70</td>
<td>210.50</td>
</tr>
<tr>
<td>S.D±</td>
<td>6.01</td>
<td>4.91</td>
<td>4.93</td>
<td>7.11</td>
</tr>
<tr>
<td>%</td>
<td>(+38.04)</td>
<td>(+42.52)</td>
<td>(+57.32)</td>
<td>(+74.36)</td>
</tr>
<tr>
<td>Liver</td>
<td>351.80</td>
<td>401.30</td>
<td>472.80</td>
<td>523.70</td>
</tr>
<tr>
<td>S.D±</td>
<td>3.80</td>
<td>7.91</td>
<td>6.11</td>
<td>6.40</td>
</tr>
<tr>
<td>%</td>
<td>(+14.07)</td>
<td>(+34.39)</td>
<td>(+48.86)</td>
<td>(+103.72)</td>
</tr>
<tr>
<td>Muscle</td>
<td>83.50</td>
<td>90.60*</td>
<td>98.30</td>
<td>108.20</td>
</tr>
<tr>
<td>S.D±</td>
<td>7.32</td>
<td>7.73</td>
<td>4.92</td>
<td>5.43</td>
</tr>
<tr>
<td>%</td>
<td>(+8.50)</td>
<td>(+17.72)</td>
<td>(+29.58)</td>
<td>(+65.62)</td>
</tr>
</tbody>
</table>

The differences between control and experimental are statistically significant (P<0.05).

* Denotes not significant with control (P>0.05).
TABLE - 31
Phospholipids (mg/g wet wt) in the organs of *C. carpio* at different periods of exposure to sublethal concentration of lead and chromium. Each value is a mean of six replicants. Percent change over the respective control is given in parenthesis.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Control</th>
<th>Lead</th>
<th>Exposure period in days</th>
<th>chromium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gill</td>
<td>11.0</td>
<td>8.2</td>
<td>6.2</td>
<td>4.3</td>
</tr>
<tr>
<td>S.D±</td>
<td>0.50</td>
<td>0.45</td>
<td>0.44</td>
<td>0.40</td>
</tr>
<tr>
<td>%</td>
<td>(-25.63)</td>
<td>(-44.20)</td>
<td>(-60.86)</td>
<td>(-68.84)</td>
</tr>
<tr>
<td>Brain</td>
<td>37.52</td>
<td>30.43</td>
<td>29.60</td>
<td>27.30</td>
</tr>
<tr>
<td>S.D±</td>
<td>1.21</td>
<td>1.01</td>
<td>0.96</td>
<td>0.67</td>
</tr>
<tr>
<td>%</td>
<td>(-18.89)</td>
<td>(-21.10)</td>
<td>(-27.23)</td>
<td>(-42.91)</td>
</tr>
<tr>
<td>Liver</td>
<td>32.40</td>
<td>29.51*</td>
<td>26.63</td>
<td>21.05</td>
</tr>
<tr>
<td>S.D±</td>
<td>1.02</td>
<td>0.96</td>
<td>0.48</td>
<td>0.72</td>
</tr>
<tr>
<td>%</td>
<td>(-8.91)</td>
<td>(-17.80)</td>
<td>(-35.03)</td>
<td>(-43.45)</td>
</tr>
<tr>
<td>S.D±</td>
<td>0.62</td>
<td>0.56</td>
<td>0.51</td>
<td>0.42</td>
</tr>
<tr>
<td>%</td>
<td>(-15.99)</td>
<td>(-28.31)</td>
<td>(-56.72)</td>
<td>(-73.00)</td>
</tr>
</tbody>
</table>

The differences between control and experimental are statistically significant (P<0.05).
* Denotes not significant with control (P>0.05).
TABLE - 32
Total cholesterol (mg/g wet wt) in the organs of *C. carpio* at different periods of exposure to sublethal concentration of lead and chromium. Each value is a mean of six replicants. Percent change over the respective control is given in parenthesis.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Control</th>
<th>Lead</th>
<th>Exposure period in days</th>
<th>Chromium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>7</td>
<td>15</td>
</tr>
<tr>
<td>Gill</td>
<td>2.610</td>
<td>3.519 4.932 4.137 5.751 3.404 2.219 3.186 2.910</td>
<td>(+11.49)</td>
<td></td>
</tr>
<tr>
<td>S.D±</td>
<td>0.104</td>
<td>0.116 0.060 0.080 0.092 0.062 0.089 0.075</td>
<td>(+34.82) (+88.96) (+58.50) (+120.34) (+30.42) (-14.98) (+22.06) (+11.49)</td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>(+34.82) (+88.96) (+58.50) (+120.34) (+30.42) (-14.98) (+22.06) (+11.49)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S.D±</td>
<td>0.520</td>
<td>0.610 0.910 0.860 0.900 0.500 0.390 0.620</td>
<td>(+49.89) (+76.40) (+89.27) (+99.07) (+49.27) (+38.75) (+28.29) (+6.00)</td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>(+49.89) (+76.40) (+89.27) (+99.07) (+49.27) (+38.75) (+28.29) (+6.00)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S.D±</td>
<td>0.319</td>
<td>0.412 0.401 0.696 0.784 0.621 0.519 0.499</td>
<td>(+18.68) (+34.77) (+48.81) (+106.56) (+46.81) (+36.16) (+43.75) (+26.15)</td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>(+18.68) (+34.77) (+48.81) (+106.56) (+46.81) (+36.16) (+43.75) (+26.15)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td>1.705</td>
<td>1.806* 1.927 2.126 2.432 1.992 1.801* 1.886 1.792*</td>
<td>(+5.92) (+13.02) (+24.69) (+42.63) (+16.83) (+5.63) (+10.61) (+5.10)</td>
<td></td>
</tr>
<tr>
<td>S.D±</td>
<td>0.009</td>
<td>0.006 0.007 0.070 0.061 0.067 0.084 0.073</td>
<td>(+5.92) (+13.02) (+24.69) (+42.63) (+16.83) (+5.63) (+10.61) (+5.10)</td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>(+5.92) (+13.02) (+24.69) (+42.63) (+16.83) (+5.63) (+10.61) (+5.10)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The differences between control and experimental are statistically significant (P<0.05).
* Denotes not significant with control (P>0.05).
Percentage change over control in the lipid content in the organs of *Cyprinus carpio* at different period of exposure to the sublethal concentrations of lead and chromium.
Percentage change over control in the free fatty acid levels in the organs of *Cyprinus carpio* at different period of exposure to the sublethal concentrations of lead and chromium.
Percentage change over control in the lipase activity in the organs of *Cyprinus carpio* at different period of exposure to the sublethal concentrations of lead and chromium.
Fig. 29

Percentage change over control in the phospholipid levels in the organs of *Cyprinus carpio* at different periods of exposure to the sublethal concentrations of lead and chromium.
Percentage change over control in the cholesterol level in the organs of *Cyprinus carpio* at different period of exposure to the sublethal concentrations of lead and chromium.