CHAPTER - 2

REVIEW OF LITERATURE
**Lead and its interactions with blood elements:**

Increasing environmental pollution has given rise to concern about the constant pressure of heavy metals on human subjects. Metals such as Zn, Cu and Fe are essential and can cause chronic metabolic disturbances if not supplemented in food (Prasad, 1976). Lead is non-essential and can cause profound haematological, biochemical and neurological manifestations in the biological systems even at trace levels. (Batuman et al., 1989; Paglieuca et al., 1990; Schwartz et al., 1990; Vyskocil et al., 1990; Dhawan et al., 1992 and Ledda Colubano, 1983). Exposure to lead pollution results in its gradual accumulation in the body and elevated levels of lead have been noticed in many tissues (Kumar et al., 1991 and Singh et al., 1993).

Tetraethyllead (organic lead), when added as an "additive" to petrol, to improve the efficiency of the engine, is a major source of wide-spread lead contamination in the automobile repair market and also inorganic lead contamination at the workplace of lead battery manufacturing units (Goldman et al., 1987; Rudolph et al., 1990 and Singh et al., 1993). There are two major routes of lead absorption which include gastrointestinal tract and respiratory tract. The absorption of lead through the respiratory tract is more complete and rapid (about 90%) than any other route (Berman et al., 1966).

Tetraethyllead (Et$_4$Pb) is dealkylated in vivo and the known metabolites are triethyllead (Et$_3$Pb$^+$) and inorganic lead in rat and man. Triethyllead is stable in rat and man, whereas in the rabbit dealkylation proceeds progressively to give inorganic lead (Bolanowska et al., 1971). Toxic symptoms following administration of tetraethyllead activity result from in vivo formation of inorganic lead. Tetraethyllead has also been shown to be converted to triethyllead in vitro studies conducted on rat liver microsomes. The reaction requires NADPH and oxygen and gets inhibited by anaerobic conditions. (Bolanowska et al., 1971)

Lead poisoning first became a notifiable disease in 1899, and during that first year 1058 cases were notified. Exposure to lead at work is now
strictly controlled, by the health and safety executive, in their mode of practice (Landrigan et al., 1990).

Exposure to lead and its inorganic compounds amounts to an increased concentration of blood lead (Kumar et al., 1991 and Singh et al., 1993). It has now generally been accepted that, under conditions of more or less constant and prolonged exposure, Pb-B reflects the quality of "biologically active" lead in the body (Willem et al., 1990). Analysis of Pb-B is, therefore, the first choice for the assessment of internal exposure of lead. Moreover, assessment of health risks due to lead exposure is generally based on the concentration of lead in blood. Blood lead, generally reflects both recent and earlier exposure (Herber, 1980).

We have (Singh et al., 1993) earlier reported in our study conducted at Chandigarh that Pb-B levels of battery and automobile workers were remarkably higher. Rudolph et al., 1990 demonstrated the persistence of occupational over-exposure of lead at many workplaces.

Rhodes et al., (1972) analysed the aerosol samples collected from different area of Texas city using EDXRF technique. The elements namely Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Hg, Pb, As, Br, Sr, Zn, and Mo were detected by these authors in the aerosol samples and observed that all the samples contained Pb and Br in the amounts correlated with vehicular pollution. Dzubay and Stevens (1973) collected the aerosol samples from St. Louis city and observed that the elements mainly S, Mn, Zn Br and Pb were found to occur mainly in smaller particles, whereas most of Si, Ca, Ti and Fe occurred in the larger particles. Caruso et al (1984) monitored the aerosol samples of Milan city using proton induced X-ray emission (PEXE) technique, and detected 16 elements. They established that the presence of Al, Si, Ca and Fe are due to natural soil dust, Pb and Br due to gasoline additive and S and V due to oil fuel.

In 1982, Oblad and Colleagues collected the aerosol samples from three different areas of Sweden namely rural, city center and industrial hall dominated by metal spraying and welding, which were studied using EDXRF.
technique. The concentration of all the elements Ti, Cr, Mn, Fe, Ni, Cu, Zn, Pb and Br) in both small (um) and large (3.5 to 18 um) particulate size were found to be minimum in rural area followed by that in city center and industrial hall.

Robinson et al. (1975) determined the molecular lead in the atmosphere and postulated that lead was first burned in the automobile and then left the exhaust as inorganic lead in the particulate form which could have fallen on the highway where it gets exposed to traffic and sun light and humidity. The elevated temperature might cause slight but significant evaporation of the lead salts, generating "molecular" lead pollutions.

The toxic effects of lead in living systems have been well documented, but its interactions with other trace elements are not fully explored. Reports are available regarding the interactions of lead with other elements (Finelli and El.Gazzar, 1977; El.Gazzar et al., 1978; Klauder et al., 1972; Kumar et al., 1991 and Dhawan et al., 1992). Lead ingestion reduces the nutritional efficiency of Zn and Cu by reducing the absorption of these nutrients (Petering, 1978 and 1980). The interactions among Pb, Zn and Cu may either retard the body growth, or even disturb the Fe metabolism (Klauder and Petering, 1977; Mahaffey and Rader, 1980; Flanagan et al., 1979 and Mahaffey, 1983). The impact of lead pollution on the level of Zn, Fe, Pb, and Br in the whole blood sample of automobile workers, lead battery workers and control populations had demonstrated that lead pollution greatly influenced the levels of these elements (Singh et al., 1992). Fe-B, Zn-B levels in automobile workers had been found to be reduced significantly as compared to control.

A mutual antagonism may exist between Zn and Pb as had been reported by Klauder et al (1972) who demonstrated that rats subjected to Pb intoxication had decreased plasma Cu levels and decreased dietary intake of Cu and increased erythrocyte Pb concentrations.

Klauder and Petering (1975) reported that when rats were fed copper deficient diets and received low level of lead (500 ppm) in diet as well,
Elevated lead levels in erythrocytes and kidney were observed. The haematopoiesis as indicated by low hematocrit and haemoglobin values was depressed significantly.

Finelli et al. (1975) reported that blood ALA-D of rats was inhibited following lead administration. These investigators showed that the reduced blood ALA-D activity could be restored in vitro by the addition of Zn.

The findings of Finelli (1975) were confirmed by Thomasino et al. (1977) who demonstrated a similar relationship between Pb and Zn in a lead poisoned patient. The patient was subjected to ethylene diaminetetra acetate (EDTA) chelation therapy to remove lead from soft tissues of the patient which caused a great increase in urinary excretion of Zn with a concomitant decrease in serum zinc and a reduction of RBC; ALAD activity. The patient, when subjected to extra Zn, resulted in restoration of its normal ALAD activity.

Cerklewski and Forbes (1976) found that lead absorption was markedly reduced when dietary Zn of rats was increased to very high levels. This was demonstrated by low blood and tibia lead. El-Gazzar et al. (1978) showed that 100 ppm dietary lead significantly reduced Zn levels in plasma, tibia and liver and that high dietary Zn (50 ppm) reduced this effect in liver and tibia.

Iron deficiency caused increased body retention of lead and increased urinary excretion of \( \delta \)-amino laevulinic acid (Mahaffey and Goyer, 1972). Several haematopoietic effects were noted in the iron deficient lead poisoned rats such as depressed haematocrits, elevated reticulocyte counts and a more severe hypochromic, microcytic anemia. The mechanism by which iron deficiency potentiates lead toxicity is not clear but there are several metabolic pathways in which iron/lead interactions could occur. For example, lead is known to inhibit heme biosynthesis at many different steps (Chisolm, 1964). Kaplan et al. (1975) suggested that these metals might compete directly for specific erythrocyte binding sites. Vanderkoi and Landesberg (1977) found that cyt-c isolated from liver mitochondria of lead intoxicated rats lacked iron. Ragan (1977) demonstrated a five fold increase in the absorption of
lead in rats when iron body stores were reduced but before iron deficiency was manifested. However, Schwartz et al., (1990) demonstrated that lead induced anaemia is produced principally by two mechanisms: impairment in heme biosynthesis and increased rate of red blood cell destruction.

It is therefore imperative that biological monitoring for lead by analysis of blood lead levels is a prerequisite for the assessment of lead toxicity in soft and calcified tissues. Further, the blood lead levels also influence the levels of other essential and non-essential elements, therefore the present perspective study aimed at quantitative evaluation of various elements in blood viz: K, Cu, Zn, Fe, As, Br and Rb in order to see the dose and time effects of lead.

In addition, it was thought to be advantageous to choose a multielemental analysis technique to have a conclusive picture of the interacting element. The technique has a large potential in biology and medicine and provides a fast reproducible and accurate means of analysis with a minimum of specimen preparation. With the advancement in experimental facilities, the values of different parameters involved in EDXRF technique, can be computed with better accuracy.

**EFFECT OF LEAD ON:**

(a)**Haematological parameters**

The toxic effects of inorganic lead had been known since ancient times and were alluded too in the writings of both Hippocrates and Pliny (Waldron, 1973). The characteristic haemotological effects of lead poisoning, such as anaemia and basophilic stippling, were fairly well established in the beginning of the 20th century. Over the ensuing years, numerous studies on the *in vitro* and *in vivo* effects of lead had shown inhibition at several sites of haem biosynthesis (Waldron, 1966; Goldberg, 1972; Albahary, 1972 and Pegliuca et al., 1990).

Lead binds avidly to the red cells with up to 50 times as much being found in the bone marrow (Albahary, 1972). Lead induced anemia is
produced principally by two mechanisms: impairment in haem biosynthesis and increased rate of red blood cells destruction (Schwartz, 1990). Lead induced anemia has been reported to occur in children at a blood lead level of 40 ug/dl (WHO, 1977).

However, no clear dose-response relationships between blood lead levels, Hb levels, total leukocyte counts and differential leukocyte counts has been established. The effects of toxic doses of lead on various blood enzymes viz: serum glutamic oxaloacetate transaminases (SGOT) and serum glutamic pyruvate transaminases (SGPT) and serum alkaline phosphatase, as a function of time also remain unelucidated.

Two transaminases (GOT & GPT) present in most mammalian tissues are known to catalize transfer of amino groups from most amino acids to form alanine from pyruvate or glutamate from a ketoglutarate (Harper et al., 1979). The serum estimation of these functional enzymes in lead toxicity would therefore, reflect the alterations in the amino acids synthesis and utilisation in different mammalian tissues. Alkaline phosphatase catalyzes the hydrolysis of various phosphate esters at alkaline pH. The serum activity of the enzyme has been reported (Harper et al., 1979) to be altered in various liver disorders and therefore has a great diagnostic value.

Hepatic functions:

Exposure to trace metals results in a variety of biochemical alterations in liver (Nicholls et al., 1984; Secchi et al., 1970 and 1971; Iannaccone et al., 1976; Dhawan et al., 1992 and Singh et al., 1993). Many of these effects are consequences of the capacity of metals to bind to nucleophilic sites in the cell, which primarily in the form of free sulfhydryl groups (Maines and Kappas, 1977).

Lead has been shown to alter hepatic transaminases and alkaline phosphatase activities (Singh et al., 1993). Flora et al (1987) reported a decrease in the activities of both glutamic oxaloacetate transaminase (GOT) and glutamic pyruvate transaminase (GPT). The decreased activity of GOT
and GPT was attributed by Flora et al (1983) to liver injury caused by lead intoxication. Boyer (1971) demonstrated that alkaline phosphatase requires Mg\(^{2+}\) ions for stability, Zn\(^{2+}\) for catalytic activities and thus an optimum Mg\(^{2+}\)/Zn\(^{2+}\) ratio is needed for maximum activity of this enzyme. Kosmider (1963) established that there is a relationship between the lead toxicity and the diminished activity of alkaline phosphatase in man as well as in experimental animals. Other workers, Conard and Barton (1978) and Sabbioni and Marafante (1976) made similar observations by reporting that lead inhibits the alkaline phosphatase activity by binding to the Zn enzyme.

We have also reported a decrease in the hepatic GOT and GPT activities following lead treatment (100mg/kg body wt.) for 3, 12 and 16 weeks, and also decrease in the hepatic alkaline phosphatase activity after 16 weeks of lead administration (Singh et al., 1993).

On the other hand, hepatic microsomal heme oxygenase is a sulphhydryl dependent enzyme which is responsible for the oxidative degradation of heme to the tetrapyrl, biliverdin (Eaton et al., 1980). Although many metal ions inhibit heme oxygenase activity, \textit{in vitro}. However, \textit{in vivo} studies have demonstrated that de novo synthesis of heme oxygenase is enhanced by the presence of metal ions including lead, which act directly on the enzyme regulatory site. Metal induction of heme oxygenase activity occurs in numerous tissues, and the capacity of different metals for induction varies between tissues. Associated with the increase in heme oxygenase activity, is a depression in hepatic microsomal cytochrome P-450 content and a concomitant decrease in mixed function oxidase activity (Maines and Kappas, 1977).

Enzyme located in the endoplasmic reticulum of liver cells protect the organism against an accumulation of lipid-soluble exogenous and endogenous compounds by converting them to water- soluble metabolites which can be easily excreted by the kidney (Eaton et al., 1980). Most compounds have to be hydroxylated first to achieve hydroxylation, the endoplasmic reticulum has at its disposal an enzymatic system, completely,
unspecific, which activates molecular oxygen for the oxidation of lipid soluble compounds. This takes place at a cytochrome (P-450), which is available in the endoplasmic membrane abundantly. The oxidation rate however is extremely slow and is dependent on the chemical configuration of the compound and on genetically determined differences of the protein moiety of the enzyme. Metabolism of drugs by this enzymatic system leads sometimes to more active and toxic compounds which produce liver injury, e.g. in the case of carbon tetrachloride (Dhawan et al., 1991).

The mechanism concerning the rise in the activity of heme oxygenase activity by metals is not fully understood. Maines and Kappas (1977) consider heme oxygenase induction to occur via a direct effect of metals on the enzyme regulatory site. While Guzelian and Bissell (1976) believed that, it occurs as a secondary effect of an increase in the regulatory heme pool derived from metal induced degradation of cyt P450 or newly synthesized heme.

Eaton et al (1980) studied the dose response effects of various metal ions on rat liver metallothionine, glutathione, heme oxygenase and cyt P450. Lead exhibited a biphasic effect on heme oxygenase activity. This biphasic effect is possibly the result of a cytotoxic effect at the highest dose which may have prevented synthesis of new enzyme and hepatic microsomal cytochrome P450 content was found to be significantly decreased with lead administration. The drug metabolizing enzyme system is complex, it has a minimum of three different components, phosphatidyl choline, a flavoprotein reductase and a haemoprotein (cyt P450). The substrate specificity is mainly provided by the haemoprotein and not by the reductase (Jenner & Testa, 1981).

Different observations had been made with variation in the routes of lead administration. Lead had been reported to show a decrease in the phase I enzymes such as cyt. P450, cyt bs, aniline hydroxylase and aminopyrine demethylase, when given intra peritoneally (Alvares et al., 1972). Further, Alvares et al (1975) demonstrated that the activity of phase-I drug metabolizing enzymes was inhibited by lead intoxication in children and
adults. On the other hand, the activity of these enzymes did not alter much when lead was administered via drinking water.

Since, lead had been known to inhibit heme synthesis (Santra et al., 1986), thereby reducing the availability of cyt P450 for drug metabolism, hence diminishing its activity. Enhanced urinary excretion of ALAD following lead administration is indirect evidence of diminished heme production (Alvares et al., 1972 & Scoppa et al., 1973). Glutathione (GSH) is a ubiquitous tripeptide which is involved in numerous cellular processes, inducing the detoxification of both endogenous and exogenous compounds. This tripeptide contains sulfhydryl groups and can effectively bind to certain divalent metals (Perrin and Watt, 1971 and Catherine et al., 1989).

Many metal ions also affect the cellular glutathione and metallothionein. Lead acetate when administered to rats by intraperitoneal injections at doses of 50, 100, 125 and 400 umol/kg/day, showed a significant decrease in the hepatic GSH levels with lower doses of 50 and 100 umol/kg/day, whereas the decrease in GSH contents at higher doses was not much evident (Eaton et al., 1980).

The drug metabolizing enzyme system represent the "mixed function oxidase", which exhibits high activity mainly in the liver (Wattanberg, 1972). The hydroxyl groups of "mixed function oxidase" system get conjugated with endogenous donor substitutes and these conjugations are catalysed by certain transferases. GSH on initial conjugation leads to the formation of mercapturic acid which may either occur spontaneously or catalysed by GST. It had earlier been established that GST serves as an important clearing agent of electrophilic products (Jenner and Testa, 1981).

Flora and Tandon (1987) had reported that when lead acetate (10 mg/kg body wt) was administered to rats daily for 8 weeks, the activities of hepatic transaminases decreased significantly, whereas the levels of glutathione were enhanced markedly. Lead induced increased level of GSH could be explained by the fact that lead must be binding with GSH in order to overcome the toxicity (Eaton et al., 1980).
Though, much has been done on the chronic and acute effects of lead on various biochemical parameters but there is enough of scope to further investigate the effects of lead alone and in combination with lithium as a function of time and dose on some of the key enzymes of drug metabolism and other hepatic functions. Further to the best of our knowledge there is no report from any other lab regarding the evaluation of role of lead on the clearance patterns of radioiodinated Rose Bengal as indicative of the alteration in hepatic functions.

**Neurological Functions:**

Exposure to tetraethyl lead e.g. by gasoline sniffing, can cause central nervous system effects, but inorganic lead compounds account for the majority of adverse effects (Marsh, 1985).

It is now generally accepted that a dose of about 1000 ppm of Pb in drinking water is the limit at which the non-specific effects of lead begin to dominate (Bornschein et al., 1977, Carmichael et al., 1981). The most important non-specific effect of lead is undernutrition. Practically, any study on lead that uses doses above this level can be considered to contain elements of undernutrition.

Recently the emphasis has shifted from external measures of administration to internal indices of exposure. These have been made to simplify the interpretation of experimental results, and to further facilitate cross study comparisons. The most important of these measures are blood lead levels. Carmichael et al (1981) reported that undernutrition induced by lead is prevalent at Pb-B levels of 100 ug/100 ml and emphasised that this level becomes a limit above which the non-specific effects of lead could be assumed to begin.

A wide variety of enzymes are known to be sensitive to lead exposure. An extensive description of the enzymatic effects of lead is reviewed in U.S.E.P.A. (1977). In comparison to several other metals, lead has a high affinity for various complexing groups, such as the imidazole cysteine...
sulfhydryl and amino groups of lysine. Lead may exert its effects either by altering the structural integrity of enzymes or by disturbing the substrate-enzyme binding (Winder and Kitchen, 1984).

The most toxic effects of lead compounds result from its effects on the brain and the peripheral nervous system. Massive entry of Pb from the blood supply into the brain is initially restricted by the blood-brain barrier or blood-nerve barrier. Stumpf et al (1980) had reported that a radioactive injection of $^{210}$Pb is initially concentrated in blood vessels and lining cells of the brain, such as vascular endothelial cells, glial cells, choroid epithelium meningeal cells and ependymal cells in some regions. Lead is absorbed and retained by the cells of blood brain barrier. Lead concentrates in brain and peripheral nervous system with time however, the concentration is in proportion to its level in blood (Goldstein and Diamond, 1973).

The enzyme system that is extremely sensitive to low lead is ALAD, the enzyme involved in the porphyrin synthesis. Both experimental and clinical studies conducted by Barlow et al., (1977) and Piomelli et al., (1980) had shown that this enzyme is markedly inhibited by lead.

Silbergeld and Lamon (1980) have hypothesized that the neurotoxic effects of lead may be due to a competitive interaction involving aminolaevulinic acid at neuronal receptors as there may be similarities between features of lead toxicity and some of the porphyrinopathic diseases (Moore et al, 1980).

The in vitro studies carried out by Selhi and White (1975) suggested that conformational changes and alteration in spatial arrangement of proteins in red cell membranes were responsible for the inhibition of Na$^+$/K$^+$ ATPase by lead at physiological concentrations. This effect was shown to be a non-competitive reversible reaction (Seigel et al, 1977) and the affinity of lead for brain ATPase was high. Neuchay and Saunders (1978) further confirmed that the site of action on the ATPase appeared to be the Na$^+$ dependent phosphorylation site, and that ATPase chelates lead. These observations have brought forth a possible biochemical basis for the oedema
of lead encephalopathy. Goldstein et al (1974) emphasized that sodium concentration in brains developing encephalopathy got increased by 88% and in contrast potassium decreased by 15%. Further, Chanez (1986) demonstrated that inhibition of Na+/K+ ATPase is an age related phenomenon, because developing brains are more sensitive to lead toxicity.

The neurochemical effects of lead in the rat had been extensively studied and reviewed (Silbergeld and Hruska; 1980). Extensive efforts were made to measure effects of lead toxicity on brain enzyme activities, transport processes, synaptic release mechanisms and neurotransmitter levels. Lead at 'low' doses had been shown to be inhibitory towards the following enzymes in the brain: tetrahydrobiopterin synthetase and dihydrobiopterin reductase (Blair et al., 1979), adenylcyclase (Goroni et al., 1979, Siegal et al., 1977), ALA-dehydrase Na+/K+ ATPase (Siegel et al, 1977), GABA transaminase (Silvergeld et al; 1972, glutaminase (Wapiney et al., 1979) and acetylcholineesterase (Louis Ferdinend, 1978).

In addition, lead also inhibits succinate dehydrogenase (Vallee and Ulmer, 1973), and alkaline phosphatase. Lead inhibits the release of AchE (Acetylcholine) from the superior cervical ganglion of the cat during the stimulation of the preganglionic fiber which was reversed by the addition of extracellular calcium (Kostial and Vouck, 1957). The cerebellum showed increased water and reduced DNA content suggesting a 15-20% deficit in cell numbers. However, DNA contents in cerebrum and RNA and proteins in both cerebellum and cerebrum areas differed marginally as compared to their respective controls, although all were consistently lowered. Michaelson speculated that perhaps interference with respiratory enzymes affected the metabolic capacity of the developing cerebellum. This speculation was further taken up and investigated by Patel et al (1974a).

They adopted the same dosing pattern as used by Michaelson (1979) and studied metabolism of radiolabelled ^14^C-glucose and 3H- acetate in the cerebrum and cerebellum (Patel and Colleagues 1974b). They indicated that although uptake of ^14^C-glucose was affected during chronic exposure, a
decrease in transamination reactions reflected a decrease in the utilization of glucose by both regions (Patel et al., 1974a). The conversion of 3H-acetate into amino acids was decreased in the lead treated rats. These observations were indicative of depression of Kreb's cycle activity in the brain. Dhawan et al (1992) reported an alteration in in vitro uptake of radio labelled nutrients (C-14 labelled glucose, alanine and leucine) in rat brain slices.

Holtzman and Hsu (1976) investigated the idea of impaired energy metabolism by lead toxicity. They studied the respiration of isolated brain mitochondria after 4% lead carbonate supplementation and observed two phases of lead effect, probably related to the increasing concentrations of lead during the first few days. Initially, during two days of lead supplementation, ADP-dependent respiration was inhibited whereas the ATP-independent respiration was stimulated. However for the next two weeks both ADP-dependent and ATP-independent respiration were progressively inhibited as indicated by the reduced activity of cytochromal oxidase, the terminal enzyme of the respiratory enzyme chain. These alterations were also observed in the cerebral hemispheres but were not significant.

Gelman et al (1979) reported a decrease in glycolytic enzyme activity in lead (225 mg/kg body wt by gavage) treated experimental rats. This disruption of energy metabolism would produce dramatic effects on the developing metabolically active nervous system.

Tandon et al (1989) described a significant increase in the dopamine (DA) contents of striatum, midbrain and pons medulla. Norepinephrine (NE) contents in midbrain and 5-hydroxytryptamine (5-HT) contents of hypothalamus, striatum, midbrain and pons medulla were raised by lead supplementation.

Although, it is possible to measure certain neuromolecular parameters in humans but the understanding of the neurological manifestations of lead at desired doses needs an experimental modeling. The administration of lead to experimental animals produced a complicated literature depending on dose,
duration of exposure or route of dosing. Therefore, the present study has been undertaken to see the effects of short and long terms lead exposures in rats. The study envisages trace elements (essential and non-essential) disposition, the alterations in in vitro incorporation of C\textsuperscript{14} labelled glucose, alanine and leucine uptake, changes in epinephrine and nor-epinephrine levels and variations in the activity of acetylcholine esterase, succinic dehydrogenase and Na\textsuperscript{+}/K\textsuperscript{−} ATPase in rat brain following lead supplementation.

**Thyroid Functions**

Thyroid functions have been shown to be altered in workers with long-term lead exposure (Hariguchi et al, 1987). They reported alterations in total tri-iodothyronine (T3), total thyroxine (T4) and thyroid stimulating hormone (TSH) in lead workers. It is generally seen that H31 uptake by thyroid in Pb exposed subjects is impaired (Underwood, 1971). Lead exposure has been reported to involve functional impairment of the pituitary adrenal axis as well as the pituitary thyroid axis. Both of these systems are particularly important to neuronal development (Sobotka et al., 1975 and Dubas et al., 1978).

Vyskocil (1990), studied the effects of lead administration (0.5% lead acetate in drinking water) for five months on hormone levels of developing rats and reported that the concentrations of non-adrenaline, adrenal catecholamines and serum corticosterone concentrations were altered but serum T3 and T4 remained unaltered.

Robins et al (1983) demonstrated that lead causes a dose-related central depression of thyroid functions. Further Refowitz (1984) found a larger negative effect of blood lead on free T4 (thyroxine) implying that his results strengthen the Robin's hypothesis that lead depresses thyroid functions. Beideman (1984) showed that lead effects thyroid indices but believed that it is the "sick euthyroid syndrome" not true hypothyroidism. Further Robins et al (1983) contradicted this observation by emphasizing that
unlike "sick euthyroid syndrome" all of the 28 patients examined in his clinical study had low T4 or EFT4 (estimated free thyroxine), and normal T3 levels by RIA.

It is therefore understood from lead intoxication literature that its toxic effects on thyroid functions as a function of dose and time are rarely studied. Therefore it is undertaken to evaluate the effects of lead on in vivo uptake of Iodine -131 in rat thyroid and estimate serum thyroxine (T4) and Tri-iodothyronine (T3) in the present investigations.

Lithium is a natural element belonging to the 1st main group of alkali metals. It is probably not an essential element but it has a role in psychiatry both as an antimaniac drug (Cade, 1949) and in the prophylaxis against recurrent attacks of manic-depressive mood swings (Baastrup et al., 1970). Lithium is ubiquitous among the nonessential elements as being one of the very few cations that are tolerated in concentrations up to 1 m mol/kg body weight.

Lithium is used in heat exchangers for air conditioning systems and as a lubricant. Lithium, hydride is used in manufacturing electronic tubes and ceramics and in chemical synthesis. The regional epidemiological studies have indicated an inverse correlation between atherosclerotic heart disease and levels of lithium in water (WHO, 1973). The presence of lithium has been verified in many plant and animal tissues and the daily intake is estimated to be about 2 mg (WHO, 1973).

The use of lithium salts offers considerable promise in the treatment of recurrent endogenous affective disorders. Lithium ion is therapeutically useful in the control of both mania and hypomania and it provides prophylaxis not only against the maniac also the depressive episodes of phasic illness (Schou, 1968). Lithium effects on the brain kidney, blood and thyroid functions have been clinically investigated in detail. Ahluwalia and Singhal 1984; Berens and Wolff 1975; and Rifkin et al; 1973].

Also, with the introduction of lithium in the treatment of manic-depressive disorders, a renewed interest has been seen in studying the
effects of this ion on various endocrine systems. Lithium treatment had been reported to cause other effects which include hypofunction inhibition of the action of antidiuretic hormone hypercalcemia with hyperparathyrodism (Christensson; 1976), stimulation of corticosterone and inhibition of gastrin release (Emerson et al., 1973). In vivo studies had demonstrated impaired, unchanged or improved glucose tolerance during lithium treatment (Shopsin et al., 1972; Vander and Gorden, 1969 and Vendsberg, 1979). However, in vitro studies had shown that lithium causes inhibition of stimulus-induced insulin release (Anderson and Blackard, 1978).

**Effect of Lithium on the distribution of other Elements**

Many studies have been undertaken to consider interactions between lithium and sodium or potassium ions (Coppen and Shaw, 1967, Hullin et al., 1968, Murphy, et al., 1969 and Baer et al., 1970). Birch (1970) however, emphasized the "diagonal relationship" in the periodic table, between lithium and magnesium and calcium ions which was reflected in terms of polarizing powers and ionic radii. Birch and Jenner (1973) observed that lithium supplementation (1 meq/kg body wt.) in experimental rats decreased brain sodium and magnesium, bone sodium and calcium and increased muscle calcium, plasma magnesium, urinary calcium and urine volume. They further observed that lithium was specifically concentrated in bones which was substantiated by Hullin et al (1968) who noticed 'episodes of lithium release' occurring over a considerable period of time in urine of a patient whose lithium medication had been discontinued, thus indicating its retention in the body.

Mellerup et al(1970) demonstrated an increase in plasma calcium concentration a short time after lithium administration and a decrease in calcium uptake in bone for upto 2hrs following the dose.

Bond et al (1975) when administered lithium chloride (30 mmol/kg dry diet) to experimental rats observed that brain had a uniform distribution of lithium throughout, comparable to that found in plasma. A decrease in the
brain sodium magnesium concentration and an increase in plasma magnesium concentration was observed.

Ghoshdastidar et al. (1989) observed a dose dependent increase of Li level in plasma, whole brain and different brain regions. The concentration of Li in whole brain and brain regions was much less than that in plasma. They also observed that concentration of Li in plasma reached a peak level at 8 h while that of Li in whole brain and brain regions reached a peak at 12 h after its administration.

These results suggested that under short-term treatment with LiCl, the clearance rate of Li in brain is much slower than that in plasma. Both single and long-term exposure of LiCl produces a dose dependent increase of Li in plasma/whole brain and brain regions.

Stolk et al. (1970) reported that treatment of rats for 10 days with rubidium caused an increase in the rate of disappearance of norepinephrine (NE) in the brain after the biosynthesis of NE was inhibited suggesting the antidepressant potential of rubidium. The rubidium contents of the control rat tissues ranged from $1.29 \pm 0.23$ mg/kg (brain) to $4.52 \pm 0.69$ mg/kg (Liver). Rubidium contents were always found to be higher in blood than in plasma.

Keeping in view the interactions of lithium with monovalent ($K^+, \text{Ma}^+, \text{Rb}^+$) and divalent ($\text{Ca}^{2+}, \text{Mg}^{2+}$) ions, an attempt has been made to see the effects of lithium on certain monovalent ions viz $k^+ \text{Rb}^+$ and divalent ions $\text{Fe}^{2+}, \text{Cu}^{2+}, \text{Zn}^{2+}$ and $\text{As}^{2+}$ in liver, blood and brain tissues of rats. The status of these monovalent and divalent ions was also seen when lithium was administered to experimental rats in combination with inorganic lead ($\text{Pb}^{2+}$).

**Lithium in haematology**

Since 1975 lithium has been utilized in the treatment of granulocytopenic conditions, including Felty’s syndrome, acute leukemia, granulocytopenia induced by cancer therapy and in the treatment of plastic anemia. Stein et al. (1977) reported lithium to have limited toxicity whereas...
Rothstein et al (1978) mentioned that because of its adverse effects, lithium should not yet be accepted as a haematopoietic agent.

Bandini (1981) reported that use of lithium carbonate during remission induction of acute lymphoblastic leukaemia inhibits granulocyte formation. During lithium carbonate treatment the main tests of PMN functions are phagocytosis, random migration and chemotaxis. Lithium Carbonate (Lc) has emerged as an effective therapy for patients with manic depressive psychosis. Occasionally, patients given this drug develop leukocytosis which is characterized by absolute granulocytosis. Jim (1979) also reported that among many side effects of lithium is leukocytosis. He presented a case history of an Korean female of 57 years, who had been taking lithium for 2 years and developed anorexia, abdominal pain, hepatosplenomegaly, WBC = 2,50000/mm³, Hb = 6.1 g/dl, differential smear segs 22%, bands 14%, metamyelocytes 26%, myelocytes 13%, eosinophils 4%, lymphocytes 4%, basophils 7% and platelet count, 1,085,000/mm³. Leukocyte's alkaline phosphatase was reported to be 15 units (normal 11 - 95 units).

There is enough scope for further studies to be carried out in the direction of time related changes with reference to the levels of total white blood cells and differential leukocytes following lithium treatment alone and in combination with lithium. The effects of lithium on serum transaminases alkaline phosphatase with short and long term lithium treatment also remain to elucidated.

**Lithium and Thyroid**

Since the discovery in 1968 that lithium treatment may affect thyroid size and function (Schou et al., 1968, Sedvall et al.,1968). This subject had been the focus of many transversal and retrospective investigations. There are reports of hypothyroidism following lithium carbonate therapy (Rogers and Whybrow, 1971). Lithium has been found to inhibit thyroid hormone release in hyperthyroid and euthyroid subjects (Spaulding et al., 1972). The possible role of lithium in this effect was indicated by the finding that protein
bound iodine (PBI) in man as well as in the rat is significantly lowered following lithium treatment (Sedvall et al., 1968, Lappaluoto et al., 1973) Dhawan et al (1985) had reported that chronic lithium supplementation suppressed serum T₄ and T₃ levels in experimental rats.

Administration of lithium is known to cause various changes in thyroid functions such as goitre, decreased serum levels of thyroid hormones and elevation of serum thyrotropin (Schou, 1976). The effect of lithium on I-131 uptake had been described variously as stimulatory or inhibitory (Fyro et al., 1973 and Mannisto et al., 1971). Inhibition of thyroidal secretion had been postulated as a major effect of lithium (Sedvall ; 1968, Berens et al., 1970, Dhawan et al., 1985).

In studies carried out earlier by other investigators, the plasma PBI level was found to be decreased (Sedvall et al., 1968) or remained unaltered (Berens et al., 1970), Mannisto et al., 1971. Lindstedt et al., (1970) had also demonstrated depressed serum thyroxine levels in patients receiving lithium therapy. Further, Berens et al (1970) and Mannisto et al (1971) had demonstrated a decreased formation of T₄ and T₃ and inhibition of iodine - 131 by the thyroid following lithium administration. Thus a great body of experimental studies supports the view that lithium reduces the level of circulating thyroid hormones by inhibiting the synthesis and release of iodothyronines from the thyroid gland.

Maarbjerg et al.(1987) demonstrated in their short-term and long-term lithium therapy in humans that T₄ (thyroxine) exhibited a small and not significant fall after 6 months and returned to the pre-lithium levels after 12 months. Thereafter, T₄ rose gradually and after 6 years of lithium administration T₄ was 53% higher than the pre-lithium level. Lithium dose was maintained so that the average serum lithium concentration remained 0.69 mmol/l during the study.

Burrow et al (1971) had shown that lithium had an inhibitory effect on the rate of disappearance of radio-iodine from the thyroid gland in hyperthyroid patients. This inhibitory effect had led to the use of lithium as
an adjunct to radioiodine I-131 therapy for thyrotoxicosis. Turner et al (1976) found that low dosage lithium therapy increased the retention of a standard with therapy dose of I-131 in the thyroid gland. Dhawan et al (1984) demonstrated that serum lithium levels remained within the therapeutic range (0.44-0.65 meq/L) when lithium carbonate was fed to rats at a dose level of 1.1 g/kg diet for up to 4 months. They further result that the uptake of I-131 by the thyroid was decreased whereas its retention ($T_b$) was increased significantly.

Dhawan et al. (1988) further showed that when lithium was supplemented to experimental rats for longer duration, the thyroidal I-131 uptake remained suppressed for up to 4 months. Further, continuing this treatment for 6 months did not result in any significant change in I-131 uptake by the thyroid. Dhawan et al (1988) also demonstrated that thyroidal biological and thyroidal effective half-life of I-131 was enhanced following lithium administration and this increase continued for up to 6 months from the 10th day of lithium treatment (1.1 g/kg diet). But to the best of knowledge no report has appeared on the combined effects of lead and lithium on the uptake and retention of I131 in rat thyroid.

**Lithium and Brain**: Lithium salts had been used since long in medicines particularly in the treatment of maniac-depressive disorders. In this respect, lithium occupies a unique position among the psychotropic drugs. However, the mechanism by which this alkali metal exerts its effect is still unknown.

Since lithium is neither protein bound nor metabolized by any means (Schou et al., 1970) its biological other biochemical role is determined by its bioavailable concentration.

Goodwin and Elbert (1973) combined patients from 10 studies and found that 81% of 413 patients showed improvements in mania during lithium treatment. There had been some double blind placebo controlled studies of lithium treatment in acute mania (Schou et al., 1969). These studies show that in most cases lithium is superior to placebo in the treatment of acute mania.
The severity of neurological side-effects can be related to serum lithium levels with the most severe symptoms occurring at toxic levels and there had been reports of neurotoxicity occurring at therapeutic levels (Van der Valde, 1971; Agulnik et al., 1972 and Rafkin et al., 1973). During lithium treatment some patients develop polyuria which usually is moderate but occasionally may amount to 6-8 liters/day (Schou, 1957, Angst et al., 1970). Polyuria can also be produced in animals by the administration of appropriate amounts of lithium (Radomski et al., 1950 and Schou, 1958a).

Mailman et al (1978) concluded in his abstract that postnatal exposure to lead induced permanent neural changes and he further documented that massive oral doses of lead caused enhancement of lithium induced polydipsia.

Recently, Ghoshdastidar (1990) reported that LiCl administration in rats caused alterations in brain catecholamine levels and further described that this action of lithium was differential and region specific. Lakshmi et al (1989), demonstrated that lithium supplementation caused neuronal depolarization by altering the neuronal Na⁺/K⁺ ATPase. He described lithium to have a biphasic effects on low affinity of the enzyme i.e. activation at low concentration and inhibition at high concentration.

Shah and Pishdad (1980) indicated that in vivo studies had shown impaired, unchanged or improved glucose tolerance during lithium treatment. However, the combined action of lead and lithium on various brain parameters viz: enzymes, catecholamines, trace metal alterations still remains unexplored.

Lithium and Liver

Lithium in general has not been considered hepatotoxic (Jefferson and Gries, 1987 and Shou, 1986). Viegut and Jeffersen (1990) described about the enquiries by Lithium Information Center about its potential in hepatitis, jaundice, cholestasis and elevated liver enzyme values. In a study (Tamura et al., 1985) on the effect of lithium on granulocyte stimulation in patients with cancer, liver dysfunctions was reported as one of its side effects.
Early studies found no evidence that lithium affects in liver (Freyhan et al., 1970). Support for lithium not being a hepatotoxin comes from the lithium intoxication literature which makes no mention of hepatic dysfunction (Amdisen, 1988; Elmallakh, 1986 and Gadallah et al., 1988). However, Winek et al (1980) presented a case report with a serum lithium level of 7.6 mmol/L and associated elevations of SGOT, SGPT and alkaline phosphatase. Further, postmortem studies (Winek et al., 1980) indicated that lithium levels were lower in liver than in kidney or brain.

Animal studies have shown variable results, due to experimental species and lithium dosages. Some studies showed no change in liver weight with lithium administration (Messiha and Dunn, 1987 and Canalty and Johnson, 1987), whereas other studies indicated a decrease in liver weights (Messiha et al., 1983 and Ibrahim et al., 1987).

Lithium affects the enzymes involved in ethanol detoxification (Ishihara, 1987). Drug metabolizing enzymes are also influenced by lithium administration. Several studies reported induction or increased activities of hepatic drug metabolizing enzymes (Parmar et al., 1974). Lithium does not appear to convert cyt P450 to cyt P450 and no change in cyt c reductase activity was found (Aniya and Matsusaki, 1983). L-alanine aminotransferase activity was reportedly inhibited by lithium administration (Kadis, 1976).

Direct effects of lithium on liver are rarely studied. The few investigations that have been done have yielded differing results. Moreover, the effects of short and long term lithium supplementation on key hepatic enzymes, drug metabolizing enzymes, trace elemental disposition and in vivo assessment of liver functions are not studied in depth.