CHAPTER - IV

DISCUSSION

DISCUSSION

The significant feature of the behaviour of lipids in the plasma of the rats under nephrotoxic insult due to uranyl nitrate intoxication is the elevation in the total lipids, total neutral lipids and total phospholipids during various phases of intoxication. Changes in the various lipid moieties seems to significant on first day of nephrotoxic insult as well as on the third day of nephrotoxic insult. The lipids seems to form an important constituent of the rat plasma. Among the various individual lipid fractions studied significant alterations were observed in the free and esterified cholesterol and triacylglycerides as well as unesterified fatty acids from neutral lipids and choline and ethanolamine phosphatides as well as sphingomyelin and lysophosphatidyl choline from phospholipids.

The present observations tend a support to the early observations of Bing et al., (1925), and Politzer (1936), and Heymann and Clark (1945), Voegtlin and Hodge (1949) and Bauer et al., (1951). They have observed hyperlipemia due to uranyl nitrate intoxication in rabbit and rats.

An examination of the thin layer chromatographic separation and the assay values of the various neutral lipids and phospholipids shows that the free and esterified cholesterol, triacylglycerides and unesterified fatty acids are

the major components of the neutral lipid, while phosphatidyl ethanolamine and phosphatidyl choline are the major components of the phospholipids. Practically all lipid fractions exhibit an increase during nephrotoxic insult.

The recent studies from our laboratory on lipolytic activity in adipose tissue (Gojer and Sawant, 1985) in kidney, liver, brain and serum (Desai and Sawant, 1985) of mice under toxic influence of nitrate suggested that a systemic disturbance of the lipid metabolism is resulted in nephrotoxic insult of animal due to uranyl nitrate. Some idea about the interrelationship of the uranyl nitrate toxicity and disturbance in lipid metabolism during nephrotoxic insult of rat can be obtained, if the nephrotoxic, hepatotoxic effects of uranyl nitrate are taken into account.

There was no gross abnormality in histopathological picture of adiposse tissue after uranyl nitrate induced toxicity (Gojer and Sawant, 1988). The early indication of nephrotoxic insult was manifested in kidney and liver with predetectable morphological changes (Desai and Sawant, 1988). These authors have suggested that chemical toxins like uranyl nitrate might be interacting with cellular energitics and interfering with the lipid metabolism leading to a net decrease in the fatty acid synthesis or accumulation of triacylglycerides as reported by Bauer et al., (1951). During early initiation phase energy demand in he toxic stress exceeds the energy source available and under such stress condition adipose tissue triacylglycerides

might be hydrolysed to release the energy in the form of free fatty acids. This has been well supported by observations on the adipose tissue triacylglycerol hydrolase activity in uranyl nitrate toxicity (Gojer and Sawant, 1988). An overall increase in the triacylglycerol hydrolase activity, during various phase of uranyl nitrate toxicity was most prevalent thing observed in adipose tissue. This enhanced lipolysis due to insufficient energy source may be one of the possibility of larger input of Free fatty acid and consequent changes in other lipid moieties leading to elevation of neutral lipid and phospholipids of the plasma. The toxic shock developed by uranyl nitrate might be causing sudden release of adrenaline form adrenal medulla, which might be directly stimulating the rate of lipolysis in adipose tissue (Wang et al., 1977).Adipose tissue, being metabolically active during the emergency might have been involved in increased brekdown of lipid content, thus increasing the level of plasma lipids in nephrotoxic insult of the animal.

The metabolic disturbance directly attributed to uranyl nitrate induced nephrotoxic insult can also be lined to the alterations in structure and function of hepatocytes and other cell organelle of the liver after uranyl nitrate intoxication. Haven et al., (1949) have reported that in rat following toxic doses of uranyl nitrate there is a tendency to develop fatty liver. In addition there is an increase in the phospholipid concentration. Recently Fukuda and Kopple (1980) have reported that, uranyl nitrate appears to have a pronounced effect on liver function. The histopathological observations by Goel et al.,

(1979) and Desai and Sawant (1987) have showed degenerative changes due to uranyl nitrate intoxication in rat liver. They have suggested that uranyl intoxication disturbs the cellular respiration affecting the energy metabolism. Disturbances in gluconeogenesis and lipid metabolism has also been suggested by Stipenski et al., (1982).

The significant changes are found to occur in liver structure and function after uranyl nitrate treatment. The elevation in the total lipid, neutral lipid and phospholipid components and the progression of hepatic lesions suggest the systemic disturbance in lipid metabolism. Uranyl nitrate poisoning of rabbit was associated with fatty liver (MacNider, 1936; Dounce et al., 1949), though there was increase in all lipid components of liver, significant elevation in neutral lipid fraction and some constituents of phospholipids was noticed (Schulze et al., 1955). The peculiar behaviour of liver lipolytic enzyme during nephrotoxic insult suggest that, hyperlipemia characteristic of uranyl nitrate induced nephrotoxic insult should not be attributed to the increased lipolysis in adipose tissue (Gojer and Sawant, 1986) and kidney (Desai and Sawant, 1988) but in additioin, the inability of liver to take up free fatty acids from blood due to fatty liver formation leading to a condition of hyperlipemia which is the consequence of systemic disturbance which have been brought about by uranyl nitrate both in adipose tissue as well as in liver.

When the problem of renal insult is taken into consideration from lipid metabolism point of view, it has been well accepted fact that, uranyl nitrate toxication results in to induction of nephrotoxic insult in animals. In rat the pattern of renal failure was characterized by progression of azotemia, alteration in hemodynamics, abnormal tubular functions (Flamenbaum et al., 1974) and increase in blood urea nitrogen (Dounce et al., 1949). In order to have a precise information about lipid metabolism in nephrotoxic insult induced by uranyl nitrate, Desai and Sawant, 1988 have worked out on triacylglycerol hydrolase activity, the enzyme being crucial in hydrolysis of triacylglycerides. During onset of early initiation phase, the triacylglycerol hydrolase activity of kidney showed an abrupt elevation. Such an increase in triacylglycerol hydrolase activity in other mammalian serum and adipose tissue under the toxic influence of uranyl nitrate has been reported by Gojer et al ., (1985) and Gojer and Sawant (1988). The immediate rise in the lipolytic activity in all organ systems like Adipose tissue, liver, kidney and brain in early initiaton phase is rather interesting. There could be a possibility of toxic shock as described by Flamenbaum, (1973) produced by local effect of uranyl nitrate. The uranium is known to produce acid, hydrolyzing the cells in that vicinity. The toxic shock thus produced might be stimulating lipotropic hormone, which in turns stimulate lipolysis in serum and other organ systems. Thus at the early initiation phase there is a significant elevation in the per

second lipolytic activity resulting in excessive production of free fatty acids and glycerol. The free fatty acids thus released in blood by kidney, liver and adipose tissue esterify cholesterol in plasma to form cholestrol esters and evoking hyperlipemia. The high quantity of esterified cholesterol observed in our present investigation supports the hypothesis of Heymann, (1942) regarding the possible mechanism of hyperlipemia in nephrotoxic insult induced by uranyl nitrate. Morin et al.,(1977) while studying the lipid metabolism in plasma, liver and adipose tissue of rats with experimental chronic nephrotic syndrome have also made similar observations. They have stated that increased plasma cholesterol and triacyglycerols may be fatty acids mobilized from adipose tissue stores.

Thus the study of lipolytic activity in rat during nephrotoxic insult have thrown light on the possible etiology of hyperlipemia produced as a result of nephrotoxic and hepatotoxic insult induced by uranyl nitrate. Hyperlipemia is a condition in which accumulation of triacylglycerides and cholesterol esters in blood takes place. Under normal circumstances the level of influx and eflux of triacylglycerides and cholesterol esters is presisely balanced by liver. Development of fatty liver condition in nephrotoxic insult imbalance the uptake of lipids obtained from dietary

sources or received from the adipose tissue metabolism. Release of lipoprotein moities from liver to blood through the synthesis of plasma lipoprotein in liver is disturbed and consequently level of various lipid fractions in blood increased leading to development of lipemia. Possibly three factors are responsible for the development of lipemia in nephrotoxic rats under uranyl nitrate toxic affect.

1 Augmentation of lipolysis in adipose tissue releasing large quantity of nonesterified fatty acids in the plasma.

2 Induction of fatty liver condition resulting in to accumulation as well as release of triacylglycrols in the liver as well as decreased activity of hepatocytes for active uptake of nonesterified free fatty acids from blood pool.

3 The toxic shock developed by uranyl nirate might be causing the adrenaline induced lipolysis in various organ systems which might be directly enhancing the rate of lipolysis and release of various neutral lipid as well as phospholipid fractions in blood.

Alterations in the level of plasma lipids in nephrotoxic insult seems to be secondary alterations. The nephrotoxic insult induce by uranyl nitrate is characterised by proteinuria, hypoalbuminia and hyperlipemia. However, these metabolic abnormalities are probably secondary to primary renal

dysfunction and hepatotoxicity . Hyperlipemia in rats with nephrotoxic insult is characterised by high plasma levels of cholesterol, triacylglycerides and phospholipids. All the lipids present in rat plasma are bound to proteins, forming various classes of liporoteins. Under normal conditions each class of lipoproteins has a specific pattern for its chemical composition. . Abnormalities in the plasma liporoteins classes in rats have been reported in fatty liver condition (Ugazio and Lombardi, 1965). The extent which proteins metabolism is affected particularly with reference to lipoprotein synthesis point of view, should also be taken into consideration.

Problems of exchange of lipid moieties blood plasma and formed elements and particularly that of red blood cells have been a subject matter of extensive studies from the point of view of lipid metabolism in plasma. Experiments carried out in vivo showed that complete exchange of red cell cholesterol and plasma cholestrol occurred with a renewal time for the red cell sterol of 8-10 hr. (Sodhi and Kalant, 1963; Bell and Schwartz; 1971). Offcourse the red cell cholesterol is in comparatively rapid free equilibrium with unesterified plasma cholestrol, but not with esterified plasma cholesterol. Hence probable role of plasma enzyme lecithin-cholesterol acyltransferase should also be taken into consideration while

discussing the problem of cholestrol exchange between plasma and red cells. There is also exists the passive equilibrium between plasma phospholipids and red cell phospholipids. It has been observed that the red cell phospholipids are involved in an exchange equilibrium with plasma phospholipids, which are primarily albumin bound. Albumin bound free fatty acid is also in rapid passive equilibrium with small pool of red cell free fatty acid. There is also active incorporation pathway for various lipid moieties between red cells and plasma. Free fatty acids and phospholipids can undergo exchange between red cells and phospholipids by process of acylation and much of the free fatty acids incorporated into phospholipids can return to the plasma as free fatty acids. There may be possibility of abnormal lipid exhcange between plasma and red cells under the hyperlipemic condition as consequence of nephrotoxic insult of rat under the toxic influence of uranyl nitrate.

In light of the above discussion, it can be suggested that the enhancement in the lipid levels of plasma during nephrotoxic insult when no much exogenous sources of dietary lipids are available, may be due to the import of products of lipolysis in the adipose tissue, liver, kidney and brain respectively. In the absence of any other possible explanation

the present suggestion can be considered favourably as it is supported by studies on lipolytic activities in these tissue in nephrotoxic insult of rat due to uranyl nitrate toxicity. If this idea is accepted, it furtehr will show how the lipid products like unesterified fatty acids and cholesterol in one place are employed in other tissues like blood and possibly to a lesser extent in liver for deriving energy or for synthesis processes. Thus, the products of lipid breakdown in the adipose tissue, kidney, liver and brain seems to be brought to the plasma where they remained accumulated as the liver is not also in functional state. Similar such situations have been observed in nephrotic insult in rat by other investigators (Marsh and Drabkin, 1960; Morin et al., 1977) as well as in fatty liver condition induced by hepatotoxic agents (Ugazio and Lombardi, 1965). This suggestion can be proved substantially if radioisotopes are employed for the study of lipid turnover in nephrotoxic insult of rat induced by uranyl nitrate toxicity.

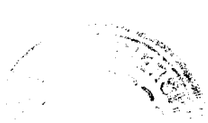
Thus, in nephrotoxic insult induced by uranyl nitrate in rat there is an overall increase in total lipids, neutral lipids and phospholipids. As a result of elevation in tricyl-glycerol hydrolase activity after the administration of uranyl nitrate from adipose tissue, liver, kidney and brain triacylglycerides are broken down and free fatty acids are liberated. These nonesterified fatty acids are ultimately brought

blood and as at the same time liver is also under the toxic stress of uranyl nitrate accumulation of these lipids takes place resulting into a condition of hyperlipemia. If the overall activity of triacylglycerol hydrolase in elevated under the toxic influence of uranyl nitrate, problem arises inspite of elevated lipolytic activity in probably all tissues and even in the serum, why there is a development of hyperlipemia ? It is a false manifestation in at least serum because blood being the transport medium the lipolytic acitvity in serum is secondary reflection of elevated lipolysis in adipose tissue, liver and kidney, ? The simultaneous, although transient elevation of the plasma lipid fractions and nonprotein nitrogen, the fatty changes of the liver and kidney, and the retrogressive changes of the kidney suggest that a systemic disturbance of lipid metabolism resulted from the uranyl nitrate intoxication. Because the rats consumed less than usual amount of food during nephrotoxic insult, during lipemic phase, the increased amount of plasma lipids is not due directly to food intake but comes rather from mainly adipose tissue and liver from the body. The simultaneous changes in profile of lipolytic enzyme triacylglycerol hydrolase in adipose tissue, liver and kidney corelates well in this concerned. Therefore, some factor(s) controlling mobilization of stored lipids is disturbed in nephrotoxic insult of rat due to uranyl nitrate intoxication. Bing et al., (1925) determined

quantitatively the lipids of rabbit kidneys and found with increase in cholesterol level. Popjak reported increase in amount of neutral fat in human kidneys. Fatty acid changes were significat. Phospholipids and cholesterol was not increased. He concluded that fatty changes of kidney represent an infiltration of renal tissues by neutral fat derived from fat depots. Dibe and Popjack (1941) produced fatty changes in kidney of rabbits by starvation. On the basis of reduction in iodine value, increase in quantity and relationship of the increase of kidney lipid to the amount of depot fat, they also concluded that the fatty acids in kidney represent an infilteration of lipids from the fat depots.

Dible and Libman produced fatty livers in rabbits by starvation and concluded that the extent of fat infilteration of the liver is determined by the amount of available depot fat, thus implying that the lipids are derived from that source. In patients with nephrotic lipemia Ahrens (1950) found a large increase of the serum neutral fat.

The changes produced in nephrotoxic rats due to uranyl nitrate toxicity in some respect similar to the so called lipid nephrosis of humans. In both, there are fatty changes of renal tubules with some chhanges of the glomeruli hepatocytes and hyperlipemia. In both the plasma lipids are greatly increased.



Thus rats with nephrotoxic insult induced by uranyl nitrate a appropriate sublethal dose provides an excellent experimental model to study the biochemical changes in plasma lipids during uranyl nitrate toxicity. As can be from the observations in the earlier chapters, this does produced excellent means to indicate morphological as well as biochemical changes during uranyl nitrate toxicity. There was found to structural as well s functional change not only in the target organ like kidney but also in adipose tissue, liver and brain ultimately reflecting effect on the plasma lipid profiles of various lipid fractions.

The augmentation in triacylglycerol hydrolase activity after uranyl nitrate administration is most significant observation of our laboratory also supported this and showed fine correlation with changes in plasma lipid profiles. A sudden elevation in the lipolytic activity after the uranyl nitrate intoxication is probably due to the toxic shock developed by the uranyl nitrate, this shock inturn might be causing release of adrenaline from adrenal medula and enhanced the lipolysis in adipose tissue, liver and kidneys, thereby releasing nonesterified fatty acids in plasma to elevate the lipid levels of various fractions. Another possible factor responsible for inducing hyperlipemic condition of plasma is the toxic influence of uranyl nitrate on liver. Under the influence of uranyl nitrate liver is found to undergo a pathogenic condition known as"fatty liver". Liver being crucial in keeping the overall lipid balance, in response to increasing triacylglycerides and other

fractions of lipids also ultimately released into blood. Conclusively, uranyl nitrate induced nephrotoxic insult seems to stimulate the free fatty acid release from adipose tissue, kidney,liver and brain due to rapid and significant increase in triacylglycerol hydrolase activity. The interrelationship between the lipemia, fatty liver and mechanism of uranyl nitrate toxic effect at cell level is an intricate problem. Study of lipid matabolism and catabolism with trace technique in nephrotoxic insult under the toxic influence of uranyl nitrate may give further information in this regard.

While concluding the present investigation on changes in lipid profile in nephrotoxic insult in rat it should be mentioned that the objectives with which present investigation was taken up, have been satisfactorily fulfilled and open some avenues for further research in the area of heavy metal toxicity. Some problems related to nephrotoxic insult and induction of lipemia which need further research are as follows :

1 Induction of lipolysis in adipose tissue and other organs have been noticed but the exact mechanism for this augmentation is not understood. As the release of adrenaline from adrenal medulla has been suggested the possible factor for this induction perhaps the study of titer of this hormone in these organs in nephrotoxic insult may give a clue regarding the induction of

lipolysis and development of hyperlipemia in plasma.

2 The development of fatty liver in nephrotoxic insult is an interesting problem. Involvement of uranyl nitrate having nephrotoxic characteristics, in the induction of hyperlipemia makes above problem complicated. Development of fatty liver, inspite of increased lipolytic activity apparantly where primary effect of seccndary cause is not known. Thus the study of etiology of development of fatty liver as compare to the other hepatotoxic conditions will be interesting problem for further research. The liver perfusion study in nephrotoxic insult under the influence of uranyl nitrate and the study of hepotocyte lipid uptake, synthesis and degradatiopn, may possibly clearify the problerm of hepototoxicity and hyperlipemia.

3 The alteration of red cell lipids in nephrotoxic insult is found to be due to elevated cholesterol levels as well as changes in some of the major phospholipids like phosphatidyl choline, phosphatidyl ethanolamie phosphatidyl serine and sphingomyelin. The study of the problem of exchanges between these lipid moities in nephrotoxic insult in red cell membrane and plasma may cast some light on derangement of red cells and shortening of red cell life span due to enrichment of cholesterol and displacement of phospholipid in red cell membrane in nephrotoxic insult. Employment of better techniques

such as gas liquid chromatography and HPLC may give further information on the fatty acid composition of various lipid constituents in rat plasma before and after nephrotoxic insult and will help us to understand the mechanism of lipolysis in adipose tissue, liver, kidney and alterations in plasma lipid profiles due to hyperlipemia.

4 One of the major vehicle for fatty acid transport in the blood is liporotein. It will be interesting to investigate the relationship between the lipids and proteins that is lipoproteins and their possible alterations in nephtotoxic insult of rat under the influence of uranyl nitrate toxicity.