C H A P T E R - IV

GENERAL DISCUSSION
AND
CONCLUDING REMARKS

Uranyl nitrate (UN), $UO_2(NO_3)_2.6H_2O$ a low radio active derivative of uranium with molecular weight SO 502 : 13 is a water soluble salt it is widely used in glass and ceramic industries for developing bright colours. It is also used in photography as good intensifier. Uranyl nitrate causes toxic effects chemically and radiologically. Uranyl nitrate is more toxic than tetravalent uranium compounds. The chemical toxicity of uranyl nitrate is attributed to changes in cellular membrane permeability by binding uranyl ion to form phosphate ligands and inhibition of glucose transfer.(Luckey and Venugopal, 1977).

In recent years uranyl nitrate is invariable used as nephrotoxin to induce experimental renal failure (Bencosme et al., 1960; Flamenbam et al., 1983). It has been used as an effective tool to induce experimental acute renal failure in different animals (Avasthi et al., 1980) Bencosme et al., 1960; Nomiyama and Foulkers, 1968; Bobey, et al., 1972).

The kidney is the principle excretery organ of uranyl nitrate and about two third uranyl nitrate is excreted in urine with 24 hours of uranyl nitrate insult. The general studies in pathology have shown that the chief organ affected by the administration of uranyl nitrate appears to be the kidney (Metcalf, 1949). Damage to the kidney is mainly in the form of tubular necrosis (Bencosme et al., 1960; Nomiyama and Foulkers, 1968) however, severe glomerular damage is also reported (Desai and Sawant, 1989 a).

The initial histopathological changes are epithelial edema, and nuclear displacement. A granular precipitate is marked in the tubularlumen. As the tubular necrosis progress, eventual loss of brushborder, nuclear pyknosis and karyohexis is reported. In severe conditions total deterioration of cellular lining, accumulation of eosinophilic amorphous material in tubular lumen is seen. Although some investigators have reported unaffected glomerulai under uranyl nitrate intoxication (Stein et al., 1975; Haley, 1982), marked glomerular nephritis or a condition analogous to focal nephritis has been reported by Desai and Sawant 1989. These changes are accompanied by peripherial leisons and increased tendency of vacuolization. The distal convoluted tubules and mendullary rays remain unaltered under uranyl-nitrate insult (Desai and Sawant 1989a).

The chief sites of deposition of uranium are bones and kidneys. However, uranium in kidney decreases rapidly while uranium in bone decreases very slowly indicating that the bone is the most important site of storage in the body (Newman, et al., 1948; Chen et al., 1961). In kidney, cortex is the essential site of accumulation with some tendency of concentration at corticomedullary junction. The selective affinity of uranyl nitrate for the tubular epithelium thereby resulting in severe renal insufficiency. The toxic injury in such animals was largely confined to the convoluted tubules resulting in a nephrotoxic insult.

Anemia is the complication of nephrotoxic insult induced by uranyl nitrate, uremia is associated with nephrotoxic insult. Hematological disorders like anemia is a classical and constant complication of renal failure. Although the relationship between anemia and uremia is not linear in non nephrotoxic insult, severe uremia is always associated with marked anemia (Callen and Limarzi, 1950). If intense anemia occurs concomitantly with only moderate uremia, another etiology should be suspected. Anemia of renal failure is normochromic and normocytic. Occasionally burr cells or helmet cells are observed. Reticulocytosis is generally low despite severe anemia (Shaw,1967). Increased 2,3 diphosphoglycerate in red cells and shift to the right of the oxygen hemoglobin dissociation curve are observed as in most anemias of other origin, implying a reduced affinity of haemoglobin for oxygen and accordingly enhanced delivery of oxygen to the tissues. The marrow is normal in the early stages of chronic renal failures but hypoplasia develops with increasing insufficiency in acute renal failure, a severe erthyroblastopoenia is offen observed.

Shortened red cell survival has been demonstrated by several investigators (Emerson, 1948, Chaplin and Mollison, 1953, Joske et al., 1956; Loge, 1958). Recently it has been shown that survival was inversely related to the degree of renal failure and uremia (Adamson, et al., 1968; Shaw, 1967). The

mechanism involved in the shortening of red cell life span is not evident. Measurements with cross transfused red cells have demonstrated the extracorpuscular nature of hemolysis in some cases (Desforges and Dawson, 1958). Anomalies in the different red cell enzymes have not been observed. However, a deficiency in adenosine triphosphate content of the red cell membrane has been described resulting in faulty transport of cations in some cases (Cole et al., 1968). Giovannetti et al., 1965 have shown that increased hemolysis and potassium loss occur if normal or uremic red cells are incubated in serum from uremia animal. This effect was significantly reduced if uremic cells were incubated with normal serum or with dialyzed uremic serum. It has been suggested that the combination of acidosis and the presence of phenolic compounds in uremia could be the cause of the alteration of red cell metabolism in human hematologic disorder with renal failure (Wardle, 1970).

It seems clear that at least two distinct mechanisms are involved in renal anemia decrease of red cell survival and depression of erythropoiesis. Generally the moderate hemolytic state in renal failure is compensated for by a normal functioning marrow.

It has been observed by several workers that there is no noticeable change in the physiology of animal during early initiation of uranyl nitrate toxicity. Peripherial blood smear at this stage showed certain morphological alterations in the red cell structure. Few reticulocytes were also noticed during

this phase. Manifestation of reticulocytes in the peripherial blood smear may be the immediate natural response of hematopoietic tissue in response the hemolytic stress and hypoxia evoked due to uranyl nitrate intoxification shortened red cell survival has been reported by Patil (1986) uranyl nitrate with its deterimental action found to be effected the total red cell mass and consequently the intracellular haemoglobin. It seems likely that, uranyl nitrate has a potential lytic effect like other metal toxins e.g. copper, cadmium and lead (Goyer and Rhyne, 1973; DeBruin, 1976; Karai et al., 1980, 1982, Keilan et al., 1984). These heavy metals are found to cause red cell hemolysis primarily by interacting with ligands,constituents of red cell lipoprotein membrane. They have suggested the proteins sulfahydril groups located in the membranes were the most favourable site of attack, other ligands including hydroxyl-phosphate and ammonia groups may also participates in complex formation with metal ions. Such interactions by altering the permeability characeristics of the membrane found to be resulting in the derangement of the ion exchange processes of the red blood cells. (Brunetti and Grignani, 1959; Rothstein, 1959, Hasan and Henberg, 1966; Jensen et al., 1974) \Fedorova, 1966 has suggested that, ligands formed with uranyl nitrate along with change in functional properties of membrane might be changing intraerythrocytory functions thereby diminishing the red cell viability (Fedorova,

1966). The active hemolysis due to derangement of red cell and change in membrane permeability has been well evidenced by Patil, 1986. He has suggested multiple mechanism for hemolysis of red cells. e.g.(i) Direct effect of uranyl nitrate ions on the red cell membranes (ii) Effect on red cell physiology by inhibiting Na^+/K^+ ATpase of the red cell membrane (iii) It may be also due to intracellular level of reduced glutathion (GSH) to values incompatable with life of red cells or it may be also due to change in the intraerythrocytotary enzymes under the toxic influence of uranyl nitrate.

Thus the development of nephrotic anemia in animals may be due to any one or all the above mentioned possibilities and its ultimate consequence is active hemolysis in nephrotoxic insult. The anemia thus developed could be entirely due to direct deterimental effect of uranyl nitrate on red cell membranes.

Lipids are the vital constituents of biological membranes. Several investigators reported alterations in lipid of the red cell membrane is the basic cause of the hemolysis (DeGier, 1964; Philips and Dodge, 1968; Cooper and Shatill, 1971; Shohet et al., 1973). Although uranyl nitrate is knwon for a long time for inducing hyperlipemia and also as a potent hemolytic agent, the exact mechanism of its action, perticularly on the red cell

membrane is not clear. Uranyl nitrate is found to induce disturbance in lipid metabolism of kidney, liver and brain (Desai and Sawant, 1988) and adipose tissue (Gojer and Sawant, 1986) and as a result of this blood being the transport medium obviously shows the alterations in lipid metabolism changes in blood cholesterol level under toxic influence of urenyl nitrate were first reported by Bing et al., (1925) and has been confirmed by Politzer (1936). Hayman and Clark (1945) reported increase in blood total lipids and cholesterol in dogs intoxicated by uranium. A more or less similar pattern of elevation of lipid levels in other mammalian species have been reported by Voegtlin and Hodge (1949), Bauer et al., (1951,1953); Schulze et al., (1955).All these reports support the fact that the uranyl nitrate has profound effect on over all lipid metabolism of the experimental animals. To confirm the etiology of hyperlipemia produced as a result of nephrotoxic insult induced by uranyl nitrate, (Gojer et al, 1985, Gojar and Sawant, 1987, 1988a, 1988b, , 1988d, 1989) and Desai and Sawant 1988, 1990 have extensively worked out lipolytic activity in various tissues of mice and rat in nephrotoxic insult induced by uranyl nitrate.

A sudden elevation in the lipolytic activity after nephrotoxic insult in rat is probably due to the toxic shock developed by the uranyl nitrate (Flamenbaum, 1973, Henry, 1968).

The interference of metal ions with the red cell membrane constituents has been recently investigated to explain the pathogenesis of nephrotoxic anemia. Although all the membranes of the red cells appear to have the unit membrane ultrastructure, their chemical composition varies greatly. Lipids and proteins are always the major components and carbohydrates are minor components. The lipid protein ratio varies. The chemical nature of the lipid components of red cell membranes also varies greatly (Nelson, 1972). Main types of lipids found in red cells are cholesterol, glycosphingolipids and phospholipids. The neutral lipids of red cells of rat consist almost exclusively of a single compound. The older data have reported appreciable amounts of triacylglycerols and cholesterol esters but our observations as well as all the recent reports do not support this. Distribution and chemistry of cholesterol in membrane has been revived nicely by Nes, (1976). In red cell membrane cholesterol seems to distributed on both outer as well as inner surface. There is also considerable variation in the distribution ranging from 1:1 to 3:1 distribution between outer and inner leaflets of the red cells (Fisher, 1976). Fisher's experiments also suggested the possibility that the distribution of cholesterol in membranes is not fixed, but varies with the ionic environment of the cells. Hemminga (1975) has suggested cholesterol in red cell membrane has ordering effect on phospholipids, it allow the coupling and

the handling on of conformational changes in membrane proteins. Alterations of red cell membrane cholesterol is another important aspect of membrane lipid metabolism. Although exchange without net transfer of cholesterol occurs when lipoprotins plasma membrane, and liposomes of cholesterol : phospholipid ratio 1:1 are inclubated togather / transfer of cholesterol from one component to another can occur if their C:P ratios are different (Kornberg and Phillips 1977). Enrichment of red cell membrane with cholesterol is found to have its effect on properties of cell. In pathological conditions causing obstruction of bile secretion, there is often an increase in red blood cell cholesterol and a much smaller increase in phospholipids so that the cholesterol : phospholipid ratio rises appreciably above 1:1 (Cooper and Jandl, (1968). This also occurs in patients lacking either the plasma enzyme, phosphatidyl choline-cholesterol acyltransferase or low density lipoproteins (Glomset, 1970; McBride and Jacob, 1970). The incorporation of extra cholesterol in to the red cell membrane can also be found to effected invitro by incubating the cells with plasma enriched in vivo with cholesterol (Sardet, et al., 1972, McBride and Jacob, 1970), and animals feed high cholestrol diets or orotic acid (Kroes and Ostwald, 1971) or with plasma to which liposomes of cholesterol : phospholipid ratio of 2:1 have been added (Cooper et al., 1975; Alderson, 1975).

In the present work there was found to be a significant increase in cholesterol. Cholesterol : phospholipid molar ratio of the red cell membrane was also significantly altered on nephrotoxic insult induced by uranyl nitrate. Cholesterol :phospholipid ration which was 0.6106 increased to the tune of 0.7496. This is increase in cholesterol :phospholipid ration remainsed at high level in second day of intoxication also. In the maintanence phase cholesterol :phospholipid ratio was also considerable high as compared to the value of control animals. It has been already observed that increased amount of cholesterol in cell is responsible for increased surface area; invitro, the cholesterol enriched cells are broad and flat wiith an irregular outline (Cooper et al., 1975). Further changes are found to occur invivo, especially in the spleen and the cells becomes spiculated and such abnormal or deranged red cells are removed more rapidly from the circulation.

The phospholipid composition of red cell membrane of rat, which is our experimental model are in excellent agreement with the other reports (Nelson, 1967, Menchaca, 1967). The major phospholipids of red cell membranes of rat are found to be phosphatidyl choline, phosphatidyl ethanolamine and sphingomyelin, Lysophosphatidyl choline was found to present in red cell lipid samples of nephrotoxic rats. The significant fact is that sum of the choline containing phospholipids is always between 50 to 60% of the total phospholipids, and the

acidic phospholipids (primarily phosphatidyl ethanolamine and phosphatidyl serine contribute the next and sphingomyelin last. The study of localization of these phospholipids in red cell membrane has not been carried out to permit any significant report, but it will an interesting area for further research.

Nephrotoxic anemia induced by uranyl nitrate in rat in the present study has indicated the red cell derangement and destruction and increased reticulocyte levels in blood. In most of the nonspecific anemias there is little or no difference in lipid composition between the anemic and normal cell populations (Zarkowsky, et al 1968 Brabac 1969) several typical anemias such as sickle cell, hereditary elliptocytosis, primary refractory and pernecious anemia also are not associated with abnormalities with red cell lipids Westerman et al., (1964) but some recently characterized anemias of nonspecific origin do involve alterations of red cell lipids (Brabec et al., 1969; Cooper, 1969).

Brabec et al., (1969) found lower than normal total lipid levels in autoimmune hemolytic anemia; the phosopholipid concentration showed no change. In newly discovered non-spherocytic anemia red cell phosphotidyl choline level has been reported to be significantly elevated Jaffe and Gottfried 1968). Cooper (1969) reported elevation of cholesterol of the red cells in a spur cell anemia, but there was no comitant increase in phospholipids. Loss of lipids in hereditary spherocytosis has been reported by Cooper et al., 1969 Cooper and Jandl, 1969 have suggested that the lipid distribution in

the red cells remain normal, and the loss of membrane lipid is due to some primary defect in the membrane, probably in the glycolytic energy system; the loss of lipid reduce the stability of the membrane, and the cells change shape and hemolyze. In acanthocytosis there is reduction in phosphatidyl choline and elevation of sphingomyelin levels (Ways et al., 1963).

Hepatic mal functions are found to affect the red cell lipids profoundly (Nye and Marinetti, 1967). These authors have observed in subjects of liver diseases a marked abnormality in red cell phospholipid distribution involving elevated phosphatidyl choline and decreased phosphatidyl ethanolamine levels. The total lipid level was either slightly elevated. Similar finding have been reported by Neerhout, (1968) adding that the red cell cholesterol was elevated and the fatty acid composition altered.

An interesting aspect of these results is that the erythrocytes in these subjects are completely normal until the liver malfunction appears. It is also interesting that cholesterol and phosphatidyl choline are primarily involved in alterations.

In the present investigation there is slight increase in total lipid and significant alterations in the levels of cholesterol, phosphatidyl choline as well as other phospholipids. Uranyl nitrate in addition to its nephrotoxic characteristics, it is also reported as hepatotoxic (Stepinski, 1982). Uranyl

nitrate toxicity is associated with fatty degeneration of liver or a condition referred to as "fatty liver" (Dounce et al., 1949). The biochemical studies performed during uranyl nitrate induced nephrotoxic insult emphasize that uranyl nitrate influence the cellular functions of liver such as energy metabolism, gluconogenesis and lipid metabolism (Stepinski, et al., 1982; Desai and Sawant ,1989b).

Thus the decrease of red cell survival in nephrotoxic insult induced by uranyl nitrate seems possibly due to alterations in the red cell membrane lipids particularly cholesterol and phospholipids. This lipid abnormality in the membrane may cause a greater sensitivity to the lytic action of reticuloendothelial system, resulting in a shortened life span of the red cells.

The second mechanism involved in nephrotoxic insult induced by uranyl nitrate leading to anemia may be insufficient production of erthropoietin leading to depression of erythropoiesis. Reticulocytosis observed as a marked event at early phase of nephrotoxic insult indicates onset of hemolytic anemia and increased erythropoietic activity as a result of hypoxic syndrome. Erythropoietin related shift of marrow reticulocytes into circulation also incdicates increased red cell destruction. However, hypochromic anemia developed may not be solely due to red blood cell membrane destruction by

uranyl nitrate because of the possibility of dyshematopoietic effect in early initiation phase /has been reported to be associated with anemia developed by certain heavy metals (Berlin and Friberg, 1960, Berlin et al., 1981).

The emergence of reticulocytes and echinocytes indicates ongoing progression of renal dysfunction. Although the fusogenic property of uranyl nitrate is not yet established, like uranyl acetate (Mujumdar et al., 1980), it may exhibit similar effect as evident in post uranyl nitrate administration.

CONCLUDING REMARKS :

While concluding the present investigation on changes in red cell lipids in nephrotoxic anemia it should be mentioned that pratically all objectives with which the present investigation was taken up, have been satisfactorily fulfilled. The present dissertation throws the light on uranyl nitrate induced nephrotoxic anemia and possible mechanism of shortening red cell life span and indicationof reticulocytosis for the present investigation standard hematological techniques and lipid biochemical techniques have been employed. The findings of present investigation gives authentic information on lipid composition of rat red cell membranes in response to the corpuscular derangement occured due to nephrotoxic anemia induced by uranyl nitrate. Both hematological parameters and

lipid analysis have been worked out in detail in order to findout the basic mechanism involved in renal anemia in relation to decrease of red cell survival and depression of erythropoiesis. Red cell structure in relation to lipid composition has been found to be greatly influenced due to toxic influence of uranyl nitrate. As there is specific toxic effects on kidney, liver and bone marrow the uranyl nitrate induced hemolytic syndrome was accompanied by a marked anisocytosis and poikilocytosis during different phases of nephrotoxic anemia.

The present investigation open some avenues for further research in the area of uranyl nitrate induced nephrotoxic anemia. Further research in this area may prove helpful in preventive and curetive treatment of uranyl nitrate toxicity. The problem which needs further research in this area are as follows :

1 The red blood cell membrane is a complex structure consisting of a proteic and lipidic fluid bilayer, with a underlying structural protein skeleton. Although many investigators have suggested asymmetrical distribution of lipids between inner and outer layer of the membrane. The biochemical basis of this asymmetry may be combined result of site specificity of the lipid exchange and renewal reactions. The physiological significance of this

lipid asymmetry under the toxic influnce of uranyl nitrate will be interesting to study.

2 Cholesterol concentration in the red cell bears a physiological significance in relation to membrane stability. Red cell cholesterol is in comparatively rapid free equilibrium with unesterified plasma cholesterol, but not with esterified plasma cholesterol. Study of the cholestrol turnover and renewal pathway with labeling experiments will give exact clue regarding its role in hemolysis under toxic influence of uranyl nitrate. Similarly the study of lecithin cholesterol acyltransferase which is responsible for the regulation of cholesterol in the cell if studied under toxic influence of uranyl nitrate will give a better picture of the mechanism of hemolysis.

3 An important property of membranes is their fluidity. Membrane fluidity is determined largely by its lipid composition, the important parameter being the length and degree of unsaturation of the phospholipid acyl chains and the ratio of cholesterol to phospholipid. Alterations in lipid and lipid ratios have been observed in nephrotoxic anemia. If the red cell membrane fluidity measurements are carried out using hydrophobic fluorescent probe and compared with values of lipids it will give better clue regarding altered membrane structure and function in control and nephrotoxic rats in uranyl nitrate toxicity.

4 An asymmetric distribution of phospholipids exist between the two valves of red cell membrane. If ATP dependent asymmetric distribution of spin-labeled phospholipids of red cell membranes are studied in relation to shape changes that occur during nephrotoxic anemia will give a clue regarding the exact mechanism of hemolytic process that occurs under the toxic influence of uranyl nitrate.

5 Metal toxicity induced deformability loss and echinocyte formation in red cells have been studied in same animals as well as in humans. The precise mechanism of uranyl nitrate induced hemolysis, however, is not completely understood. Metal complexation with neighbouring sulfhydryl groups in membrane and subsequent oxidation to a disulfide bond have been suggested as a possible cause of membrane damage leading hemolysis. Inhibition of various glycolytic and membrane bound enzymes by copper ions has been also implicated as causitive factor for corpuscular derangement and echinocyte formation in certain metal induced hemolytic anemia. It will be interesting to test this mechanism for uranyl nitrate induced hemolysis by studying levels and activity of glycolytic and membrane bound enzymes in rat under nephrotoxic insult induced by uranyl nitrate.

6 In recent years uranyl nitrate is invariably used as nephrotoxin to induce experimental nephrotoxic insult leading to uremia. The manifold clinical symptoms of uremia in nephrotoxic insult induced by uranyl nitrate suggest a generalized toxic effect on essential cellular functions such as membrane alterations, disturbances in energy production or transport processes. An appropriate approach to demonstrate such alterations of cellular functions may be the study of electrolyte transport of red cells. It will be intresting to study uremic or nephrotoxic red cells with respect to intracellular concentrations of sodium and potassium under toxic influence of uranyl nitrate.

Thus it can be concluded from the above much more work is necessary to clarify the mechanism of hemolysis and reticulocytosis in nephrotoxic anemia induced by uranyl nitrate especially in regard to structure function corelations of the red cell membrane.