C H A P T E R - I

INTRODUCTION

1 RED BLOOD CELLS AND ANEMIA

The red blood cell is uniquely biconcave in shape, and designed for one primary function to deliver oxygen to tissues in adequate quantities. In functional sense, the red blood cells as a solution of haemoglobin contained within a membrane, with sufficient metabolic machinary maintain the cell in an to operating state, for 100 to 160 days depending on the species. The diameter of red blood cell averages 6.3 μ in new born rat, number ranges from 2.5 to 3.5 millian red cells per cubic.mm.

Their mature red blood cell is composed chiefly of water 65% , and Hb 33% constitute more than 95% of its total protein. Remaining constituents are lipids, carbohydrates, phosphorus, various metals like zinc, copper etc. cations, vitamins, other organic compounds such as urea, uric acid, creatine, creatinine etc. The red cell contains all enzymes required for aerobic and anaerobic catabolism of glucose.

Intracellular metabolic pathways that takes place in red cell generate first ATP, required for the maintenainance of red cell membrane function, and integrity, and also for synthesis of number of compounds. Secondly NADPH which prevents the denaturation of haemoglobin, and lastly NADH maintains heme iron in the reduced functional state.

The red cell has low metabolic rate and no energy stores. Glucose is degraded to lactic acid via Embden-Meyerhof's pathway and energy is released in the form

of ATP, used to maintain cell shape and controls Na, K flux and thereby the osmotic pressure of the cell. In this pathway for every molecule of glucose there is possible net gain of two ATP molecules. About 90% of glucose is utilized by this pathway. Active cation transport ceases in absence of ATP, cell swells and hemolysis ensues. With ATP deplition cell becomes rigid, and unable to transverse the microcirculation and membrane fragmentation results. In case of rat ATP levels are lower than mouse and man, NADH and NAD are also generated through above said pathway.

Remaining ten or less than ten percent of glucose is metabolized through hexose monophosphate shunt, results into formation of carbon dioxide and phosphorylated pentose sugar, which by a series of molecular rearrangement turned to Embden-Meyerhaf's pathway. It results into generation of NADPH, as on cofactor in glutathion reduction, reduced glutathion prevents the oxidation of sulfhydryl group of haemoglobin molecule

In addition to this the Rapaport-Leubering shunt also takes place in red cell results into formation of 2,3 DPG or 3-PG. Normal RBC have high concentration of 2,3 DPG, it has high affinity towards haemoglobin, and binds readily with haemoglobin. In presence of 2,3 DPG affinity of haemoglobin to oxygen sharply lowered. In case of rat there are high levels of 2,3 DPG. Ferrous iron atom in heme binds riversibly with

oxygen, resulting into formation of oxyhemoglobin, so as to keep the heme iron in functional state by reducing Fe^{+++} . NAD dependent methaemoglobin reductase pathway also found in red cell where diphorase enzyme convert methaemoglobin to functional haemoglobin.

The physiological mechanism of red cell death is not completely understood. It is well established, however that under normal circumstances death occurs with remarkable regularity after a definite period. The total number of circulating red cells is the result of the interaction of the rates of red blood cell formation and red blood cell distruction.

Decrease in the circulating mass of red blood cells results into anemia. It may results from decreased generation of these cells or their premature destruction or loss through haemorrhage.

The procedures are established for evaluation of weather it is regenerative or non-regenerative, or it may be due to abnormalities of maturation of red cells.

In regenerative type of anemia, the erythroid marrow responds to the decreased red cell mass by accelerating erythropoisis. It results in reticulocytes, macrocytosis, polychromacia, nucleated red cells, Howell-jolly bodies, besophillic stippling, leukocytosis in peripheral blood. This

type of anemia is caused by either blood loss or hemolysis.

Blood loss can be acute or subacute or chronic. The character of peripheral blood picture varies markedly, the degree of response depending on the severity and rapidity of blood loss.

When the anemia is due to hemolysis, then the life span of red cell decreases and results into, existance of hemolytic state. In this decrease is balanced by increased erythopoiesis, there is full compansation and no anemia occurs. When decreased red cell survival is not balanced by increased erythropoiesis results into hemolytic anemia. There are two main factors that results into increased red cell destruction, i.e. intracarpuscular defects and extracarpuscular defects.

In intracarpuscular defects, red cell survival is shortened in the affected animal and in a normal recipient transfused with the defective cells.

In extracarpuscular defects, the red cell servival is reduced in the patient. The hemolytic factor (s) is part of the environment that the red cells encounter in the circulation of the anemic animal. Normal red cells also have a shortened life span when transfused into the affected animal.

In both of above types the defective or damaged red cell may be lysed or fragmented, lose membrane or undergo

coagulation, necrosis of a portion of its content. If the damage does not results in immediate distruction, it leads to elther a rigid deformed cell, or as symmetrical spherocyte. The abnormal red cell, is destroyed through an interaction of the red cell and RE cells. The process of red cell phagocytosis is highly developed in spleen.

Severe membrane damage causes the spherocytosis and/or rupture of the red cell with release of its content into circulation. Intravascular hemolysis, while small amount of haemoglobin may simply deplete the haptoglobin-hemopexin systems, producing hemosiderinurid large amounts are associated with overth hemoglobinemea & hemaglobin-urea and very severe causes can cause renal damage.

The destruction by fragmentation of red cells, in the circulation results in both reticuloendothelial phagocytosis of pieces, and intra-vascular lysis, with deplition of plasma haptoglobin and hemopexin methemalbumin appears in the circulation and hemosiderin found in the urine.

The compansatory increase in erythropoiesis in hemolytic anemia occurs after a lag period of a few days. If hemolysis is sudden in onset and phagocytic in type there may be only rapid drop in the hematocrit and hyperbilirubinemia. Red cell production in hemolytic anemia is higher than in other anemias. Presumaly because the catabolized red cells provide the best

source of iron.

In second type that is in non regenerative anemias the erythroid marrow is unable to produce enough red cells, to replace those lost by normal attrition. This can results from a deficiency of factors necessary for red cell production that is iron, toxic inhibition by chemicals, infection, neoplasia or a deficiency of erythropoietic stem cell.

In third type that is anemia due to abnormalities in maturation, erythropoiesis have been increased but not accompanied by increase in the number of reticulocytes in the peripheral blood. On the basis of cell size and Hb concentration, anemia is of macrocytic, hypochromic, microcytic, but never hyperchromic.

Anemia is also one of the complication of renal failure. Anemia of renal failure is normochromic and normocytic. Reticulocytosis is generally observed in anemia associated with renal failure. At least two distinct mechanisms are involved in renal anemia : decrease of red cell survival and depression of erythropoiesis. Insufficient production of red cells is evidently the main mechanism, as the general hemolytic state observed in renal failure would easily be compensated for by a normal functioning marrow. Shortened red cell survival in nephrotoxic anemia has been demonstrated by Patil et al., (1986), Gojer

and Sawant (1992). However, the mechanism involved in the shortening of red cell life span is not evident.

METAL TOXICITY

Several trace metals are known to essential to life. The other metallic elements are non-essential in a biochemical sense but they are exceedingly important to modern industry. It is a sad fact of life that the heavy industrialisation of the past few decades has been accompanied by health problems due to toxic effects of certain metals. Metal compounds are used in pesticides, raw materials, catalysis or energy are brought up as resides in food, water and air. Excessive absorption of these residues results into hepatotoxicity, renal insufficiency or billiary obstruction lead to the disturbance of homeostatic mecanisms and ultimate accumulation of these metals in tissue level cause toxic effects. These toxic metals tend to change biologic structures and systems into irreversible and inflexible confirmations leading to deformity and death.

Metal toxicity in mammals results in early mortality, growth retardation, impaired reproduction and depression of physiologic parameters. At cell level derangement of cell membrane permeability and antimetabolic effects. Most of the metals interact with proteins or with unknown compounds leading to a change in physiological processes. Changes in rates of

catabolic decomposition of essential metabolites, enzyme inhibition and irreversible conformational changes in macro-molecular structure. The increasing use of heavy metals as well as rare earth metal compounds in different fields has stimulated the interest of studying toxicology of these metals.

3 URANYL NITRATE TOXICITY

Uranyl nitrate, the rare earth metal derivative has gained much attention because of its wide industrial use and its hazard of inducing renal failure (Flamenbaum et al., 1973). It is widely used in industry mainly in glass and ceramic industry. It is good photographic intensifier and is used in photographic industry. Solubility of uranyl salts is the dominant influence on their toxicity and growth retardation, renal dysfunction and consequent toxicity. The progression of uranyl nitrate toxicity is marked by symptomatological changes in animal behaviour and concomitant weight loss (Gojer and Sawant, 1986). Kidney is the target organ system in uranium toxicity. Estensive work has been carried out on renal pathophysiology and histopathology (Yano and Ueshima, 1982; Flamenbaum, 1983; Desai and Sawant, 1988). Kidney and bones have been reported to be the major sites for accumulation of uranium salts (Ferriti et al., 1951). Uranium in kidney decreases rapidly whereas the uranium in bone decreases very slowly indicting that the bone is the most important site of the storage in the body (Tannenbaum and

Silverstone, 1951). The general route of excretion of uranyl nitrate in all animals appears to be by a way of urine. Roughly two third of uranium was found to be excreted via urine (Neuman et al., 1948; Tannenbaum and Silverstone, 1951).

4 NEPHROTOXIC INSULT AND URANYL NITRATE

Uranyl nitrate is found to induce acute as well as chronic renal failure. Azotemia and calcemia with severe renal insufficiency and renal tubular damage has been observed in dogs, rats and rabbits due to uranyl nitrate intoxication (Bencosme et al., 1960; Nomiyama and Foulkes, 1968). Due to renal injury levels of plasma proteins altered and appearance of catalase and phosphate occur. Cytotoxic effect of uranyl nitrate at tubular level results in the decrease in insulin clearance. Histopathological changes in kidney after uranyl nitrate induced acute renal failure include proximal tubular destruction (Ryan et al., 1973). Proximal tubule is the main site of necrosis in uranyl nitrate induced renal failure.

5 UN TOXICITY AND BLOOD

The development of severe renal insufficiency during uranyl nitrate induced nephotoxic insult is marked by remarkable changes in total Red blood cell count (Patil et al., 1986; Gojer and Sawant, 1989, Giglio et al., 1989). Uranyl nitrate seems to have a potential lytic effect like salts of other metals such as copper, cadmium and lead. Uranyl nitrate by binding with red cell membrane found to alter the functional properties of

the membrane and intra-erythrocytary functions (Gojer and Sawant, 1989) which found to diminish the red cell viability consequently resulting in decreased red cell count. Alternate explanation for marked anemia in severe stages of acute renal failure seems to be either active hemolysis or impaired erythropoiesis. The severity of renal damage and depression in erythropoietin production after uranyl nitrate induced nephrotoxic insult has been recently reported by Giglio et al., (1986; 1989). Thus alleviation of renal erythropoietic factor due to subsequent tabular and glomerular damage may be responsible for the manifestation of hypochromic macrocytic anemia marked during progression of uranyl nitrate induced acute renal failure. Agranulocytosis manifested during the pathogenic progression of acute renal failure is the most common manifestation of drug induced hemopoietic damage (Pisciotlad, 1971). this condition is analogues to aplastic anemia. The lymphocytosis is also marked during pathogenic progression which is attributed to the natural defensive reaction in response to necrotic degeneration (Gojer and Sawant,, 1989). Significant elevation in the serum lipolytic activity is observed to be remarkable event during the uranyl nitrate induced acute renal failure (Gojer et al., 1985).

6 LIPID COMPOSITION OF RED BLOOD CELLS

The mature red cell is composed chiefly of water and haemoglobin. The remainder consist of numerous enzymes and coenzymes; other proteins, predominately constituents of the stroma, lipids carbohydrates, phosphorus, sulfure, zinc, copper, lead, tin, manganase, aluminium, silver, cations, anions, vitamins and other organic compounds such as urea, uric acid, creatine, creatinine, phosphorylated nucleotides. The fully mature, non-nucleated red cell contains no DNA and RNA. It is incapable of replication and cannot synthesize haemoglobin or its components. Nonetheless; the red cell takes part in a number of complex metabolic activities. Red cell stroma consist primary of a lipoprotein complex; the red cell has an orderly physicochemical structure and expend energy to maintain its integrity and discoidal shape.

The lipid in the mature red cell of mammals is solely contained in the plasma membrane. Lipid account for nearly fifty percent of the mass of the membrane while proteins are also present in a similar amount. Only small quantities of sugars in the form of glycoproteins and glycolipids are present although these more hydrophilic moieties may be particularly important in the immunologic specificity of the red cells.

The lipids are responsible for approximately one-half of the mass of the red cell membrane. Phospholipids and unesterified cholesterol account for the vast majority of these lipids and are both present in nearly equal propertions (Sweeley and Dawson, 1969; Nelson, 1972). Small amounts of glycolipids, especially "91-4" are also present. Of the phospholipids, phosphatidyl - choline, phosphatidyl - ethanolamine and sphingomyelin are the major lipid fractions, while phosphatidyl serine, phosphatidic acid and phosphatidyl inositol as well as very small amounts of neutral lipids are also present. The large mass of these fatty acyl groups in these membrane phospholipids probably influences the physical and permeability characteristics of the membrane to a significant extent, and changes in their composition may be expected to affect changes in those characteristics. In particular, an increase in either the chain lengths or the saturation of the hydrocarbon chains would tend to decrease membrane lipid mobility in comparison to membranes with shorter or more unsaturated fatty acids. However, membrane free cholesterol, which is intercalated between the phospholipids may tend to equalize these differences to some extent. There are characteristic patterns of esterified fatty acids within each phospholipid class which have been noted in several mammalian species as well as in human red cell (Nelson, 1967).

The lysophosphotidyl choline is found to be esterified on glycerol backbone with one fatty acid. They are present in red cells in only small quantities, they play important role in the renewal pathways of membrane phospholipids. They may be of similar importance in relation to stability and permeability characteristics of the membrane (Shohet and Haley, 1973). Phospholipids with two fatty acids are highly lypophilic, lysophosphatidyl cholines are balanced in terms of lypophilic and hydrophilic characteristics. They, therefore, tend to distribute at phase interfaces. This change involving solubility increases both their detergent qualities and their rate of exchange between the cell membrane and the plasma (Tarlov, 1966). Because of these qualities, lysophosphatidyl cholines in low concentrations can lyse red cell membranes. In even smaller concentrations they can produce profound, eventually irreversible changes in the shape of the membrane outline. The smooth biconcave contour of the cell changes progressively to a slightly crenated and heavily spiculated cell under the influence of progressively increasing amounts lysophosphatidyl choline. Brecher and Bessis (1972) have formed this process of change in red cell as echinocytogenesis. Eventually there is microvesiculation and membrane loss producing irreversible prelytic spherocyte forms.

Though several theories have been proposed for the anatomic localization of the lipids within the membranes, their precise disposition is still not known. Gorter and Grendel (1925) have originally proposed that a large percentage of the lipid is arrayed in the biomolecular leaflet. Danielli and Davson (1935) have advanced this model and suggested that in the bilayer disposition, the polar head groups of each lipid layer face away from the centre of the membrane into the hydrophilic environment of the cytoplasm and the plasma, while the long acyl tails of the lipids form a central hydrophobic core of the membrane. This hydrophobic core is in a liquid crystalline state of normal temperatures and may facilitate the physiological essential flexibility and deformability of the red cell membrane. Singer and Nicolson (1972) have proposed that many of the protein element of the membrane inserted into this lipid matrix are much like iceberges floating in water. In this model some of the proteins and glycoproteins are confined to one leaflet while others, especially those assumed to have transport and shape-mediating roles, span the membrane. In addition, both the inserted proteins and the lipids are free to move laterally within the plane of the membrane at comparatively rapid rates. However, motions across the bilayer from one leaflet to the other are probably much more restricted. Since it is reasonable to assume that the lipid is a viscous determinant in the membrane,

any significant changes in lipid composition that affect the membrane's internal microviscosity might be expected to have an effect on the flexibility of the whole cell. However, the protein constituents of the membrane are of major importance in this regard and calculations of the physical forces involved suggest that lipid protein interactions must be much more important than pure lipid effects (Rand, 1968).

The lipids are not symmetrically distributed between the inner and outer leaflets of the membrane. From the series of ingenious labeling experiments, it appears that the majority of the phosphatidyl-ethanolamine and the phosphatidyl-serine are contained within the inner or cytoplasmic leaflet of the membrane, while the majority of the phosphatidyl-choline and the sphingomyelin are contained in the leaflet facing the plasma (Bretscher, 1972; Gordesky and Marinetti, 1973). The biochemical basis of this asymmetry may be the combined result of the site specificity of the phospholipid exchange and renual reaction, and the sluggish lipid exchange rates between the inner and outer membrane leaflets. Although the full extent of the physiological consequences of this asymmetry are not known, it has been suggested that there is a considerably transmembrane charge potential induced by the excess of positive charges on the inner leaflet. The asymmetric distribution of the typical intramembranous particles has been demonstrated by the freeze-

fracture electron microscopy (Pinto da Silva, 1972), where the large majority of particles are found on the inner face of the cleaved membrane. This may be due to consequence of lipid asymmetry.

Normally red cell is incapable of de novo lipid synthesis. It is well endowed with mechanisms for lipid turnover and renewal as well as mechanisms for lysophosphatidyl detoxification. Both these mechanisms are probably important for modulating red cell function and regulating red cell life.

Several investigators have reported the evidences of abnormal red cell lipid turnover associated with membrane instability i.e. hemolysis. (Snyder, 1977). In severe hepato-cellular liver disorders red cells may become laden with excess cholesterol and to a lesser extent phospholipids (Neerhout, 1968). Initially they assume a "targeted morphology" on air-dried blood smears due to an increase in membrane surface are induced by the excess cholesterol. Using the cell's resistance to colloid osmotic lysis as an indirect measure of surface area, it has been calculated that the surface area varies directly with the membrane cholesterol in ratio of 1:4. Thus, for a forty percent increase in membrane cholesterol there will be an increase in membrane surface area of ten percent (Cooper and Jandl, 1968). In the usual case of mild hepatic disease this morphological curiosity is of little important. However, in severe

hepatocellular disease an apparant exaggeration of this process producing a massive accumulation of cholesterol in excess of phospholipid in this membrane coupled with a secondary effect of "processing" the clumsy cholesterol laden cells within the reticuloendothelial system results in spur cell anemia (Cooper, 1969; Cooper et al., 1974). Under these circumstances the red cell survival is markedly shortened and the red cell morphology is extremely bizarre with "spurred" membranes covered with spikelike projections (Smith et al., 1964; Silber et al., 1966). The appearance of these cells in the circulation is a serious prognostic sign.

The increased cholesterol content appears to be induced by some abnormality in the usual equilibrium of plasma lipoprotein cholesterol with that of membrane cholesterol. The actual plasma levels of cholesterol are not necessarily high and the presence of abnormal lipoprotein or an increase in surface active bile acids have been invoked as hypotheses to explain the change in the plasma red cell cholesterol equilibrium (Cooper and Jandl, 1968; Seidel, et al., 1969). Moreover, decreased activity of cholesterol acyltransferase (Gjone and Norum, 1970) has also been observed in this disorder and would further favour cholesterol accumulation in the red cells.

Whatever the underlying cause of the increase in the cholesterol in the cell, this certainly seems to be the primary

cellular abnormality responsible for the anemia. It is as if a great excess of cholesterol disorders the membrane organization, causing either heaped domains or weaknesses within the previously ordered organization of the lipids, so that the spikes are formed. The precise secondary mechanism of the anemia is not defined for spur cells, but from the bizarre shape and increased viscosity of the cells it is likely that the spurred cells are trapped in the microcirculation of the spleen or other reticuloendothelial organs (McBride and Jacob, 1970). Splenic sequentration has indeed been found in some cases (Silber, et al., 1966). Further evidence for this mechanism can be inferred from studies on human red cells in spur cell disease (Cooper, 1969). Transfusions of ^{51}Cr-labeled normal cells into these patients found to show the labeled Cohort gained surface membrane as measured by increased osmotic resistance in the first 24 hr after infusion. Subsequently, however, they become more fragile, with increased osmotic fragility and eventual premature lysis (Cooper,1969). This development suggested a mechanism of progressive loss of available membrane by either the spicule formation itself or by membrane lipid loss consequent to removal of the fully formed spicules in the patient's reticuloendothelial system (Shohet, 1972).

High concentration of certain phospholipids have been found to be one of the basic cause of membrane abnormality in

certain anemia. Such anemia has been referred as high phosphotidyl-choline hemolytic anemia. The basic cause of high concentration of phosphatidyl-choline is found to due to the defect in active incorporation pathway. Abnormality in red cell phospholipid composition is found to be associated with increased cation permeability of the membrane (Jaffe and Gottfried, 1968; Shohet et al.,1971). Lipid analysis of the red cell showed significant elevations in phosphatidyl choline and reductions in phosphatidyl ethanolamine. In these cases there was no abnormality in cell cholesterol and incontrast to spur cell disease, there was no abnormality in the passive exchange of cholesterol between plasma and red cells. However, when fatty acid incorporation studies were done on these cells, distinct abnormalities in activities were detected. There was marked increase in gross incorporation of fatty acid into phosphatidyl choline in these cells, while incorporation into phosphatidyl ethanolamine was decreased. The ultimate effect was found to be abnormality in phospholipid composition. This is in turn results into an abnormality in membrane function found for these cells whereby both sodium and potassium permeability was increased. The clinical significance of hemolytic anemia in these cases may be related to the changes in the phospholipid composition of red cells.

It has been proved beyond doubt that heavy metals such as copper, cadmium and lead have potential lytic effect on red cells. (Goyer and Rhyne, 1973; DeBruin, 1976; Karai, et al., 1980, 1982; Kielan-Bak et al., 1984). These pertinent observations have shown that these metals have specific effects on red cell membranes. They cause hemolysis primarly by interacting with lipoprotein constituents of the red cells by forming ligands. The protein sulphahydryl groups located in the membrane is the most favourable site of attack. This protein-metal interaction is found to alter the permeability characteristics of the membrane invariably resulting into derangement of the cation exchange functions of the red cells (Brunetti and Grignani, 1959; Rothstein, 1959; Hasan and Hernberg, 1966; Jonsen et al., 1974). Toxic effect of lead on red blood cells have been studied extensively (DeBruin, 1976; Arena, 1976). Under the toxic effect of lead abnormal red cells are seen in the peripherial circulation. Bishop and Surgerner (1964) have reported that survival time of red cells is decreased due to lead toxicity because of binding of active site by metal ions and altering the permeability characteristics of the red cell membrane. There is specific effect on potassium permeability and inhibition of active transport due to ATpase dependent Na^+ - K^+ transport. The ultimate effect of which results into shrinking of red cells due to loss of potassium and water.

Mercuric ions are found to cause agglutination and hemolysis of red cells. At low concentration Mercuric salts are found to block glucose entry in red cells by complexing with phosphate ligand. Copper ions are also found to cause red cell deformaties by gradually transforming the red cells into poikilocyte and echinocyte. (Ito & Kon, 1987).

Uranyl nitrate a rare earth metal is used as nephrotoxin to induce acute and chronic renal failure (Flanenbaum 1972a; 1972b Avasthi et al., 1980). It also brings about morphological changes in many organs. Kidney and bone marrow are the two main target site of this metalotoxin (Gojer and Sawant, 1987; Desai and Sawant, 1987). Our laboratory has been actively engaged in extensive work on toxicity of uranyl nitrate. Uranyl nitrate causes industrial health hazard similarly its potency in inducing acute renal failure has been acknowledge by physiologist in the area of nephrotoxicity as the rate of mortality by renal failure is very high and the experimental acute renal failure model of rat is analogus to human acute renal failure, its detailed study is very much essential.

Generalized changes in serum lipids under toxic effect of uranyl nitrate have been described by Sagare and Sawant (1980, 1981). As the alteration in the lipid metabolism during toxic effect of uranyl nitrate was noticed the study on lipid

storing depot was brought into focus. Acute toxicity of uranyl nitrate and response of triacyl glycerol hydrolase activity in mice adipose tissue was cleared out (Gojer and Sawant, 1985a, 1985b).

The tolerance developed by experimental Rats and mice to repeated doses of uranyl nitrate and its relationship with cell repair process has been worked out (Gojer and Sawant, 1986). Patil et al., (1986) have described hematological observations under toxic effects of uranyl nitrate. The pathophysiology of kidney, liver and brain under the toxic effects of uranyl nitrate have been reported by Desai and Sawant (1985, 1988). After establishing the experimental model for study of acute renal failure (Gojer and Sawant, 1986) various antidotes such as saline loading, dithiothreitol treatment have been tried (Gojer and Sawant, 1988, 1992). The synergestic effect of water diuresis, dithiothreitol and dopamine have also been described. Recently effects of dithiothreitol has been tried as potent antidote for recovery of red cell structural derangement (Kulkarni et al., 1990). They have reported that on the 48 to 72 hr. of uranyl nitrate toxicity red cells are transformed as echinocytes and shows the presence of cytoplasmic bridges between them. Bone marrow morphology of Rat in toxic influence of uranyl nitrate has been described by Kadam et al., (1990). The corpuscular derangement nephrotoxic insult induced by uranyl

nitrate is the most interestingobservation in our laboratory (Gojer and Sawant, 1990; Garware et al., 1990; Gojer and Sawant, 1992).

Reasons That Led To The Present Investigation

The above critical review of uranyl nitrate toxicity clearly indicates that toxification of uranyl nitrate, which has long been strirring up several physiologist and drawing their attention due to its clinical importance. Hemolytic hypoxia induced by uranyl nitrate has been drawn much attention towards resultant changes in red cell structure and its correlation with ongoing prognosis of acute renal failure. An early indication of renal insufficiency as early as 48 to 72 hrs post uranyl nitrate administration, has been reported by subsequent alternation in red cell structure (Meola et al., 1982), which could be used as a sensitive diagnostic tool for early acute renal malfunction .

Thus though the extensive data is available on uranyl nitrate toxicity, biochemical changes induced by uranyl nitrate toxicity, histopathological manifestations in kidney, liver, brain and bone marrow (Sawant and Gojer , 1993), the mechanism of hemolytic anemia due to nephrotoxic insult of uranyl nitrate is either not worked or little is knwon about it.

PLAN OF PROPOSED WORK

Keeping in mind all above aspects and crucial factors which show the importance of the studies on behaviour of red cells in uranyl nitrate toxicity, it was proposed to find out

possible mechanism of nephrotoxic anemia. the proposed work hence aimed at :

1 Since uranyl nitrate is potent nephrotoxin, its effects have been concentrated on renal structure and function and to some extent on the liver, there is very little information on its exact effect on red cell structure and physiology. Hence it is planned to study lipid profile of red cells under toxic influence of uranyl nitrate.

2 Mechanism of action of metalotoxins including uranyl nitrate is yet contraversial. It will be interesting to study the effect of uranyl nitrate on structural integrity and permeability proporties of red cells.

3 Heavy metals like Cadmium, Copper, Lead, mercury are known to induce hemolytic anemia due to abnormal membrane of red cells. It will be interesting to observe whether anemia induced by uranyl nitrate is also similarly due to hemolytic interaction, forming ligands with red cell membrane proteins, or due to change in the lipids, or due to abnormal erythropoiesis.?

4 The morphological alternations in red cells is the characteristics feature of metal toxin induced corpuscular derangement, whether the changes brought about in red cells in nephrotoxic insult in uranyl ntrate toxicity are sequencial ? Can we use this criteria of structural

alterations in red cells as a sensitive indicator of uranyl nitrate toxicity.

5 The echinocyte formation is characteristic phenomenon in uranyl nitrate toxicity (Gojar and Sawant, 1992). The basic cause of this membrane abnormality is either cholesterol or phospholipids ?

6 The problem of assembly and breakdown of cellular membranes has most often been approached on the assumption that lipid-lipid and lipid-protein interactions are the major forces of membrane cohesion. It will be interesting to study the lipid profiles of the red cell membranes and changes in it to find out the change in the structural integrity of membrane and dependence of lipid-lipid or lipid-protein interactions as they are the major forces of membrane cohesion.

7 An asymmetric distribution of lipids has been observed between the two valves of red cell membrane (Opden Kamp , J.A.F.1979). this asymmetry particularly with reference to cholesterol and phospholipids is disturbed under the nephrotoxic effect of uranyl nitrate causing the hemolysis. It will be interesting to observe the alterations in cholesterol and phospholipid concentrations during nephrotoxic insult of Rat due to uranyl nitrate intoxication.

The present study has been carried out into three different phases of nephrotoxic insult viz.

1	Early initiation phase	1 to 24 hr.
2	Late initiation phase	24 to 48 hr.
3	Maintenance phase	48 to 72 hr.

The dose of 5 mg/kg body weight of uranyl nitrate has been selected for proposed work. For the present work, standard and accepted hematological biochemical and analytical techniques have been used. Werever essential the possible modifications were made in existing techniques to get correct and authentic data on hematological and biochemical events occurring during uranyl nitrate induced nephrotoxic insult in rat.