CHAPTER IV

GENERAL DISCUSSION AND CONCLUDING REMARKS

During the onset of early initiation phase (2,4 hrs) of uranyl nitrate (UN) induced toxicity, there was a considerable effect on total hematology of the experimental animal. UN with its detrimental action, affected the total red blood cell mass and consequently the intracellular hemoglobin to a considerable extent. On the basis of pertinent observations, it seems likely that, UN has a potential lytic effect like salts of other metals, such as, copper, cadmium and lead (Goyer and Rhyne, 1973; DBruin, 1976; Karai et al., 1980, 1982; Kielan et al., 1984). These metals are reported to have a potential lytic effect on RBC membrane. These heavy metals cause hemolysis primarily by interacting with ligands, constituents of erythrocyte lipoprotein membrane. Although the protein-SH group located in the membrane is the most favourable centre of attack, other ligands including hydroxyl-phosphate and ammonia groupings may also participate in complex formation with the metal ions. Such interactions while altering the permeability characteristics of the membrane, invariably result in the derangement of the cation exchange functions of the red cell (Brunetti and Grignani, 1959; Rothstein, 1959; Hasan & Hernberg, 1966; Jensen, et al., 1974). In a similar manner, UN forming ligands with RBC membrane probably alters the functional properties of the membrane and intraerythrocytory functions, which may consequently diminish the red cell viability resulting in decreased RBC count (Fedorova, 1966). This is evidenced by a consequent hemolysis at 0.4 and 0.6 % saline concentrations. Some analogus findings regarding the reduction of erythrocyte life span associated with other metal poisons like

cadmium, molybdenum and arsenic are reported by Berlin and Friberg (1960); Robinson et al. (1969). The lysis of the red cells on exposure to metal salts could be due to multiplicity of mechanisms like (1) direct effect of UN on integrity of RBC membrane forming ligands with - SH group (Rothstein, 1959; Brunetti and Grignani, 1959; Jensen et al., 1974). (2) In some way, possibly through the inhibition of the Na^+/K^+ ATPase of the membrane, the mechanical fragility of the red blood cells could be increased by exposure to UN. (3) It may be invariably associated with a primary fall in the intracellular level of reduced glutathion (GSH) to values incompetable with life of the cell (Fegler, 1952; Pernis and Magestretti, 1960). (4) The functional parameters expressive of the integrity of erythrocyte membrane, such as osmotic resistance due to increased intracellular volume. Such effect has been detected during cadmium, mercury and lead poisoning (Ponder, 1937; Swensson, 1959; Hoffman and Eden, 1958; Vincent and Blackburn, 1958; Rand and Burton, 1963). (5) Defect of membrane permeability such as to derange the normal monovalent cation exchange, are clearly evident in the action of lead (Vincent & Blackburn, 1958; Grigarzik & Passow, 1958) and cadmium, mercury (Joyce et al., 1954; Vincent and Blackburn, 1958). (6) Like other metal toxins such as lead and arsenic, UN may have its detrimental effect on pump mechanism associated with accelerated loss of K^+ which may further lead to alternation in the osmotic resistance of the cell and subsequent loss of red blood cells due to lysis. (7) Intraerythrocytory enzyme change in hemolytic conditions due to UN, may further

result in marked depressent effect on hemolysis (Fedorova, 1966; Fairbanks, 1967).

Thus the development of macrocytic hypochromic anemia in the early initiation phase of UN toxicity could be due to various above mentioned possibilities. The hypochromic anemia developed could be entirely accountable to the direct detrimental effect of UN on red cell membrane. Since the possibility of dyshematopoietic effect in early initiation phase would not be feasible, although it is reported to be associated with anemia developed by certain heavy metals (Berlin, 1960, 1961). However, the possibility of dyshematopoietic effect in the late initiation and maintenance phase should not be overlooked.

In response to lytic effect of UN on RBC membrane the erythrocyte sedimentation rate was accelerated in this phase. The condition of accelerated ESR is prevelently seen in many infections and inflammatory conditions; or in local conditions like, effect of UN hydrolysing to produce HCl and thus producing a local inflammation (Dounce et al., 1949), may lead to the consequent acceleration of ESR in the early initiation phase of UN induced toxicity. The pathophysiological conditions thus initiated in this phase are evidenced by relative increase in blood urea nitrogen level and tri-acyl-glycerol hydrolase activity of UN treated animal in this phase. These parameters have been used previously to detect the pathogenic severity of the animal under the influence of UN by Gojer and Sawant (1986).

With a higher dose concentration (10 mg/kg) there was

again a substantial decrease in RBC number and Hb concentration. There should have been greater effect of UN in early initiation phase at this dose concentration; but interestingly enough experimental animals exhibited lesser declination than the early dose of 5 mg/kg UN. However, a substantial decrease in RBC number, Hb concentration and accelerated ESR rate with mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) are indicative of macrocytic hypochromic anemia characteristic of UN treatment. Other metal toxins like lead and mercury exhibit a typical normocytic hypochromic anemia (Saita et al., 1952; Hutchinson & Stark, 1961).

Although the general appearance of the animal varies according to dose and time, there was no significant behavioural change observed at this dose concentration in the early initiation phase. The animal looked physically normal under the effect of both concentrations of UN. However, irrespective of physically well being of the experimental animal there were definite hematologic changes in the animal in this phase. The anemic condition thus initiated in this phase was latter on manifested physically in the fully developed pathogenic condition. The etiology of anemia under both concentrations of UN could be the same and there is very little space for consideration of effect of UN on major erythropoietic tissue like bone morrow or on a precursor, renal erythropoietin, since animal requires more time for deposition of UN in bone marrow and kidney (Chen et al., 1961); the effect of UN on RBC membrane attacking membrane integrity either forming ligands or increasing

mechanical fragility appears to hold good in the early initiation phase of UN induced toxicity.

The white blood corpuscles show a subsequent decrease in their percent values in response to both low and high dose of UN in the early initiation phase. This decrease is probably be due to effect of UN, diminishing white blood cell vaibility. Similar effect of some toxic drugs like amidopyrine, chloromycetin, thiouracil, resulting in leukopenia are reported. The decrease in total WBC count is mainly due to the loss of monocytes and neutrophils. The mechanism by which UN attacks the WBC viability remains obscure. The hematological values in this phase support the macrocytic hypochromic anemia showing gradual increase in MCV and considerable decrease in MCH and MCHC.

In the late initiation phase the pathogenic condition shows a gradual progress under the influence of both low and high dose of UN, as evidenced by a gradual rise in blood urea nitrogen level and the serum lipolytic activity. The progression of pathogenic condition in the late initiation phase could be due to the attack of UN on vital organ systems. In this phase of UN toxicity, most of the UN administered is absorbed in blood forming a diffusible bicarbonate complex and a non-diffusible albumin complex (Dounce *et al.*, 1949). The diffusible bicarbonate complex enters in cell interior of the organ systems like liver, kidney and bone marrow. As reported earlier, most of the UN administered accumulates in kidney and bone marrow (Chen *et al.*, 1961). Uranyl ions have been shown *in vitro* to combine

to form reversible and stable complexes with phosphoryl and stable complexes with phosphoryl and carbonyl ligands on membrane surface and inhibit sugar phosphorylation and transport (Luckey & Venugopal, 1977). Thus producing its inhibitary effect on cellular energetics and the ultimate effect of which is cell necrosis or degeneration (Fedorova, 1966; Fairbanks, 1967). In the late initiation phase the process of cell degeneration is evoked by diffusible bicarbonate complex of uranyl ions while the nondiffusible counterpart, i.e. albumin complex attacks on the blood corpuscles and the blood formed elements. Thus in the late initiation phase, accumulation of uranyl ions in kidney and bone marrow leading to consequent necrosis and the circulating uranyl ions affecting blood formed elements evoked severe pathogenic condition as compared to the early initiation phase, as can be seen from elevated blood urea nitrogen level and serum lipolytic activity in this phase and naturally a concomitant alterations in the hematological picture are seen in this phase. The subsequent loss in red blood cell could be again attributed to the lytic effect of UN, as evidence by increased hemolysis at 0.6 and 0.8 % saline concentrations. The structural and functional impairement of RBC result in declined intracellular hemoglobin concentration and accelerated erythrocyte sedimentation rate.

The etiology of macrocytic hypochromic anemia observed in late initiation phase could be the structural and functional impairement of RBC due to UN attack. However, in this particular phase, UN probably exhibits a dual action, a direct injury to peripheral red cell and a destructive or suppresive effect on

erythropoiesis. Whether anemia thus developed is due to an effect on developing bone marrow or on the membrane of the circulating cell is obscure. But there is every possibility of impaired renal erythropoietin secretion due to renal cell necrosis or impaired erythropoiesis, due to untoward effect of accumulated UN on bone marrow. It is apparent that the formation, maturation and release of erythrocyte into the blood by bone marrow is governed by equilibrated forces that maintaine the red cell mass with oxygen demand, since there was considerable loss in RBC number in early initiation phase, there should have been increased production of erythrocytes from bone marrow to commensurate the decreased number of RBC's in circulating blood. However, animal shows again a persistant state of macrocytic hypochromic anemia in the late initiation phase. So, possibly the ability of bone marrow to give birth to new erythrocytes is probably affected by UN in the late initiation phase and thus RBC's failed to attain a normal level and the anemia persisted in the late initiation phase.

As the late initiation phase progresses into fully developed maintenance phase the blood urea nitrogen level and the serum lipolytic activity show maximum elevation. This the phase, at which the necrosis of the liver and kidney is clearly manifested (Flamenbaum, 1973; Desai and Sawant, 1984). The histopathological effect of UN at this phase reaches to its peak and the affected organ system loose their functional ability and as a result of which, animal shows unhealthy physical and behavioural changes. In this phase, animal becomes limp and

inactive, they are cold to touch. They are responseless to touch and sound, they show lowered body temperature. Animal survives in this morbid state for 2-3 days more and latter on dies in coma. An overall effect of severe pathogenic condition is reflected in hematologic picture of the experimental animal. The RBC's and hemoglobin concentration shows a prominent loss in their percent values as compared to the earlier two phases of UN toxicity. The probable reason for this increased loss could be the effect of UN on membrane integrity or on the life span of RBC's, in addition the involvement of other factors like renal dysfunctioning due to cellular degeneration affecting the humoral erythropoietic agent, erythropoietin, or deterioration of bone marrow cells, destroying the developing erythrocytes should be equally considered. Erythrocyte sedimentation rate exhibit relative acceleration in response to the total hemolysis. Changes in differential analysis, include loss of neutrophils and monocytes and little increase in lymphocytes, basophils and eosinophils is probably the most common manifestation during chemically induced hemopoietic damage and many drugs have been reported to be associated with it (Pisciotta, 1971) this condition could be referred as an analog of aplastic anemia, a condition at which, bone marrow elements are greatly affected. Basophilia and eosinophilia is relatively a non-specific finding and occurs in a number of seemingly unrelated disorders. Hence the significance of these elements in different phases of UN toxicity is complex.

The hematological picture under the prolonged toxicity test of UN exhibited symptomatological change in blood formed elements. The total RBC count showed a subsequent fall in their percent values, the variation in dose concentration however, had a very little effect. As result of decreased RBC count, intracellular Hb concentration declined relatively and likewise erythrocyte sedimentation rate. As compared to the alterations in the hematological values in acute toxicity test, the prolonged toxicity test of UN gave less significant but consistant alterations.

The loss of RBC's and consequent loss in Hb concentration in prolonged toxicity of uranyl nitrate, casts better light on mechanism of action of uranyl nitrate on the blood. As the experimental animal is consistantly exposed to a repeatative dose of UN, a sustained pathogenic condition is evoked in the animal body. This pathogenic condition was not severe as compared to the acute toxicity test. This can be marked with very little elevation in blood urea nitrogen level and serum lipolytic activity. There was repeatative attack of UN on RBC membrane, since the concentration of UN was low enough; the lytic effect of UN could have been neutralised to some extent by initiation of erythropoietic regulatory mechanism in the animal body. Thus giving lesser effect on total RBC mass and hemoglobin concentration. This could be supported by the comparative MCH and MCHC during this test. Amongst the leukocytes, total WBC number was found to be moderately affected by prolonged effect of UN. Lymphocytes show little increase while neutrophils

decline considerably. The basophils and eosinophils remain unaffected. The effect of UN on leuckocyte varied according to the dose concentration.

The behavioural and physical observations in the animal exposed to prolonged treatment of UN showed no abnormal or undesirable effects. The animals looked physically well, with no symptomatologic effects of UN in contrast to the acute toxicity test of UN. The acquisition of tolerance to smaller dose concentration of UN in prolonged toxicity test could be the possible phenomenon, which ameliorated the effect of UN on blood corpuscles and blood formed elements. The possibilities of such acquisition has been reported by Gojer and Sawant (1985).

The retardation of coagulation time or hypocoagulation observed in both acute and prolonged toxicity test of UN is a common finding in experimental metal poisioning. Hypocoagulation after an experimental intoxication with lead is reported by Cataldi and Odaglia, 1957; Moreo and Candura, 1960; Saita, 1960; Sroczynski and Kossmann, 1967; Danilove, 1968; Alferova, 1971. Heavy metal salts like mercury, cadmium, silver are also known to affect fibrinolysing, causing depression of coagulation mechanism (Worowski, 1961, 1967, 1968). In aluminium intoxication hypocoagulation is reported by Waldron et al., 1971. Reduced coagulability of the blood has been reported in experimental arsenite poisoning arising from reduction of several prothrombin converting factors amongst which are factor V, VII, X, IX (Moreo and Candura, 1960; Facchini, et al., 1959; Barni et al., 1957).

Since blood clotting is the final outcome of series of successive reactions, the conversion of prothrombin to thrombin is essential, which proceeds through two different pathways, i.e. the intrinsic and extrinsic routes, both require the participation of series of substances with thromboplastic activity. Clotting then follows from the action of thrombin upon fibrinogen. The metallic toxins which affect the normal coagulation offer various possible reasons behind this coagulatory disorders. Most of the rare earth metals are stated to have potent anticoagulant properties (Dyckerhoff et al., 1936; Vincke and Oelkers, 1938) and the precise mechanism of action of most of these agents is not clear. In case of UN intoxication with present observation, it is very difficult to comment upon the nature of action of uranyl ion on various factors involved in the blood coagulation. However, hypocoagulation under the toxic influence of UN is remarkable finding which may help in the early diagnosis of metal poisoning.

CONCLUDING REMARKS :

By concluding the present aspect of uranyl nitrate toxicity and its effects on blood corpuscles and blood formed elements, it should be mentioned that practically all objectives with which the present investigation was taken up; have been satisfactorily fulfilled. Thus, the present dissertation describes in detail the effect of uranyl nitrate, with varying dose concentrations and duration, on the hematological picture of the experimental animal along with few biochemical parameters such as blood urea nitrogen and serum lipolytic activity, taken up as an index of pathogenic

severity of the animal in acute toxicity test as well as prolonged toxicity test. For the present investigation suitable hematological and biochemical methods have been employed, modification were made, wherever needed, with in the standard techniques to achieve correct data. The present investigation gives reliable and authentic information on the effect of uranyl nitrate on blood corpuscles and blood formed elements during the acute toxicity test, in addition it encompasses the effect of UN during prolonged toxicity test, which not only gives a better idea about the nature of UN toxicity in the animal body but also offers a better understanding of pathophysiological mechanisms involved during UN induced acute renal failure. The information on which may be helpful in a preventive/curative therapy of UN induced ARF. A phased programme study of UN induced ARF avails a thorough understanding of the biochemical alterations that take place in blood and consequent effect of them on blood formed elements.

The present investigation opens several avenues for future research on UN induced toxicity and experimental acute renal failure. Some idea of such future work which might throw a better light on hematological manifestation after UN administration are listed below in brief. -

(1) UN seems to have effect on all blood corpuscules and blood formed elements, a condition referred to as pancytopenia, although a illdefined term, a specific reason for such an effect of uranyl nitrate on blood corpuscles needs further investigation.

(2) UN is known to accumulate in bone marrow and kidney and evidences of which are extensively documented. To know the exact etiology of anemia produced, it is indispensable to study the effect of UN at bone marrow level, a possibility of bone marrow deterioration should be probed and effect of such deterioration on developing RBC's should be extensively studied.

(3) Like bone marrow, kidney is the another target organ system which offers site for UN accumulation. The effect of accumulated uranyl nitrate on renal functioning and thereby its effect on renal erythropoietic prescursor, erythropoietin, deserves further investigation.

(4) Since spleen is known as a graveyard of RBC's, the histopathological studies of spleen may help in understanding the fate of damaged RBCs during UN induced toxicity. The phased programme study of histopathology of spleen under UN administration may cast better light on the etiology of anemia induced by UN.

(5) The effect of uranyl nitrate on leukocyte production is not well understood. In what way UN destroys WBC's, right from the early initiation phase is inexplicable. The term leukopenia being poorly understood, the present aspect needs more specific research about leukogenesis during UN induced acute renal failure.

(6) Changes in differentials analysis during various phases of UN toxicity, although are significant, for a pertinent

interpritation of their peculiar alterations, role of these cells in the experimental ARF should be worked out.

(7) Acute damage to red cell or its hemoglobin content can result in an impairement of oxygen transport with consequent hypoxia. The sign and symptoms in such case are due secondarily to damage to the central nervous system and/or heart, the organ most sensitive to hypoxia. The study of these organ systems under UN toxiciation may add to our understanding of pathophysiology of experimentally induced ARF.

(8) Meola et al. (1984) have reported a clumping of RBC's and formation of bridges between discoid erythrocytes, 48 hrs. after the UN administration. Study of RBC's under UN attack at higher magnification and the chemical nature of the intercellular bridges and the possible reasons for the formation of these bridges during UN induced toxicity may give better clue for early diagnosis of UN induced acute renal failure.

(9) Hypocoagulation is a prevalent phenomenon observed in most metal intoxication studies. The mechanism by which the metal salts effect the normal coagulation is heavily documented. However, very little is known about the toxicity of UN and the factors involved in the process of hypocoagulation which deserves further investigation.

To make an end is to make a beginning
The end is where we start from

- T.S. Eliot.