CHAPTER I

A REVIEW OF WORK ON URANIUM TOXICITY

A N D

PLAN OF PROPOSED WORK

INTRODUCTION : I) METAL TOXICITY

In the modern era, toxicology has evolved as multi-disciplinary field of study; definations of toxicology often reflect the area of study from which the defination is derived. Toxicology is broader than the more parachial definations. It is more than the science of poison. Further, the discipline of toxicology is still in its most rapid evolutionary stage and a proper defination must include it breadth and take account of its probable future development.

Basically, toxicology is a study of the adverse effect of chemical compound upon biological system. Toxicity is the capacity of chemical agent to adversely effect activity of living organism, its growth, health, life span and reproductive capacity. Early mortality, growth retardation, impaired reproduction with mortality of offsprings, neoplasm and chronic disease symptoms are some of the common criteria for toxicity in mammals. Adverse effects also includes behavioural changes in individual animals and ecological changes that effect collective population.

The word 'toxic' may be considered as being related with regard to the adverse or harmful effects of chemicals. Certainly many chemicals are so non-selective in their action on tissues or cells, they may be said to exert an untoward effect on living organisms. Furthermore, such chemical may be effective in rather small concentrations. In contrast to this, a given chemical compound may be sufficiently selective in its ability to produce

harm, that it acts only on specific cells. A chemical may be harmful to essential systems present in several species of organisms, but capable of exerting its harmful effect only on a few of these species, because of protective device present in resistant species. Hence it is more resonable, as experience has shown to recognize that the degrees of harmfulness, degrees of safeness for any chemical, even the most innocuous of substance when taken into body in sufficient amounts may lead to undesirable; if not distinctly harmful effects. On the other hand, the most harmful of all chemical compounds can be taken into the body in sufficiently small amount, so that there will be no undesirable effect for such chemical. It is apparent that the harmfulness or safeness of chemical compound is related essentially to the amount of that compound that is present in the body. Therefore, if the term toxicity is used; it is indispensable to identify the biological mechanism on which harmful effect is produced.

An understanding of what toxicology is may be gained by considering the practitioners engaged in this field. The activities and contributions of toxicologists are many and varied. The most obvious role of toxicologists in the field of biomedical area is concerned with intoxication by the drug and other chemical and the demonstration of the safety or hazards of the drug prior to their entry in the market. The recognition, identification and quantitation of relative hazard from occupational or public exposure to toxicants comprise other major function.

This relates closely to private and governmental responsibilities to assure safety of workers and general public in their contact with industrial and commercial products; in ensuring air and water purity, as well as safety of food, drugs and cosmetics. The assessment of hazard of such widely used material as pesticides or fertilizers is also the responsibility of the toxicologists, on the other hand, the development of such poisons with a selective action on weeds, insects and other unwanted organisms is also the province of toxicologists.

M.J.B. Orfila (1814-1815), a Spanish physician is known as a founder of modern toxicology. He was the first to attempt a systematic correlation between chemical and biologic information of the known poisons. Much of his contribution is based on the personal observation, effects of poisons in several thousand dogs. Among other contributions, he singled out toxicology as a discipline distinct from other and more broadly defined toxicology as the study of poisons (1818). Orfila (1814-15) also focused attention to problems combining chemistry and other allied subjects. He pointed out necessity of chemical analysis for legal proof of lethal intoxication and divided methods for detecting poisons, some of which are still used. A major out come of his activity was the emergence of the analytical approach to autopsy material for purpose of detection of accidental and intentional poisoning. The introduction of this approach survive in modern toxicology as one special area; that of forensic toxicology.

The era of modern toxicology poineered by Orfila marked

the beginning of a number of analytical developments that made poisoning detectable. Although this analytical gaint step was of paramount importance; it should not obscure the development of investigational sophistication in world of toxicant. Francois magendie (1783-1855) spent a significant part of his effort in a study of mechanism of action of emetine and strychnine. He was drawn to the arrow poisons used by the native and began a study of their actions. Louis Lewin (1920, 1929) was a prodigious figure in toxicology. He dealt with the toxicology of methyl and ethyl; and higher alcohols, chronic opiate effects, effects of hallucinogenic materials in plants and chloroform. Development occured rapidly in 20th century. On the other hand, there were many toxic and therapeutic agents that served as a starting point for fundamental study for mechanism as with the development by Peters and et al. (1945) of dimercipol (BAL) as an antidote to arsenic containing gases and studies of mechanism of BAL action on organic arsenical by Carl Voegtlin et al. (1924). On the other hand, there were developments leading to discovery and understanding of toxic substances for use by man such as discovery and study of DDT by Paul Muller (1946) and the discovery and development of organophosphorous insecticide by Schrader (1952).

Modern toxicology is a multidisciplinary science and it borrows freely from several of basic sciences. A knowledge of; and an ability to study the interaction between chemicals and biologic mechanism is predicted upon a background in all of the

basic physical, chemical and biologic subjects. Toxicology borrows freely knowledge from chemistry and more particularly biochemistry. It is dependent upon the knowledge of statistics and public health is also fundamental to the study of toxicity. Pathology is said to be a part of toxicology, for a harmful effect from a chemical agent on a cell, tissue or organism must necessarily be disclosed itself in the form of gross microscopic or submicroscopic deviations from the normal. The field most closely related to toxicology is pharmacology, for pharmacologists must understand not only beneficial effect of chemicals but also harmful effects of those chemicals that may be put to therapeutic use. Thus toxicology is defined as the study of harmful action of chemicals on biologic mechanism, which comparises many areas of service and research e.g. environmental toxicology is a branch that deals with the incidental exposure of man and other animals to harmful contaminants of the environment; Forensic toxicology deals with medical and legal aspects of the adverse effect of chemical on human. Behavioural toxicology is a new concept introduced by Mello (1975), Spyker (1975). Clinical toxicology deals with the study of symptomatologic effects of chemicals. Experimental toxicology studies the effect of toxic levels of chemicals and drugs. Industrial toxicology deals with material involved in occupational health hazards and industrial hygine. Economic toxicology is a study of agent such as insecticides, pesticides, herbicides and defoliants and their effects on pest, domestic animals and humans.

Modern industrialization has introduced many harmful metals

into the environment by redistributing them from mobilized area and minerals. Metal salts have been used therapeutically in human and veterinary medicine for centuries, but man and animals are now exposed to more metal salts than they were previously. Metal salts used in pesticides, raw materials, catalysts, or energy end up as residue in food, water and air, metals for which there are no nutritional requirements may react with biologic systems to cause considerable effects. Excessive absorption leads to the break down of homeostatic mechanisms and to the accumulation of metal in tissue levels high enough to cause toxic effect.

Literature on the toxicity of metals for mammals has been thoroughly reviewed and pertinent information is summerized and interpreted. New concepts have been introduced. Classic compartments of knowledge have not been respected, for example nutritional, stimulatory, therapeutic and toxic effects of metals are presented as components of single continum of complete dose response concept. Understanding of the physiological and chemical basis of metal toxicity in mammals will be useful as a guide to present practice and to future research, legislation and regulation. In absence of specific toxicity data about some trace metals, these generalization should provide 1) the base for predictive toxicity, 2) guidelines for safety and 3) the motivation to obtain more complete toxicity data. In order to understand the basic reactions possible between metals and other biochemical constituents; the hard and soft acid and base theory of Pearson (1963) is reviewed. This leads to a better understandi[illegible] of

co-ordination, chelation, ligand formation, and metal interaction with proteins, nucleic acids, carbohydrates and lipoproteins of cell membrane and organelles.

As cited before, criteria for metal toxicity in mammals are early mortality, growth retardation, impaired reproduction with mortality of offspring, depression of physiologic parameters, neoplasm and chronic disease symptoms. At the cellular level derangement of cell membrane permeability and antimetabolite activity are the effects of metal toxicity. Metal can interact with protein, leading to a allosteric effect or with DNA or RNA to stop normal metabolism or with unknown compounds, leading to a change in physiologic processes to a change in behaviours or even to a change in physiological and ecological systems. Changes in rates of the catalytic decomposition of essential metabolities, enzyme inhibition and irreversible conformational changes in macromolecular structure are some of the effects of metal toxicity at molecular level. Biological and environmental variations influence the toxicity of metals. The inherent toxicity of metals and its compound in biologic systems depend on the electrochemical character and oxidation state of the metal, absorption and transport of the metal in the body tissue, the stability and solubility of its compounds in body fluid, its ease of excretion and its reaction with the functioning tissues and organelles and with essential metabolites and other metals.

Toxic effects are also dose dependent, the effect of a

metal can be categorized as dose that cause 1) no symptoms or detectable effects, 2) stimulatory effects, 3) therapeutic effects, 4) toxic or harmful effects and 5) death.

In general, all toxicity testing methods can be divided into two categories, first category consists of those tests that are designed to evaluate the general overall effects of compounds on experimental animals. The individual test in this category differ from each other, basically in regard to duration of the test and the extent to which the animals critically evaluated to general toxicity. These tests are identified as acute, prolonged and chronic toxicity test.

ACUTE TOXICITY TESTS :

The single test that is conducted on, essentially, all chemicals that are of any biologic interest is the acute toxicity test. The test consists of administering the compound to the animal on one occasion. The purpose of the test is to determine the symptomatology consequent to administration of the compound and to determine the order of lethality of the compound. This necessitates selection of a route of administration, preparation of the compound in a form suitable for administration by the selected route and selection of appropriate species. For the present investigation, the acute toxicity tests are performed on rats. Because of their low cost, their availability in lab and the fact that abundant reference toxicologic data for most compounds are available for this species. Regardless of the species selected, all test animals should be in state of good health and should be observed, for a period of time in laboratory. The sequence

of determining the acute toxicity of any compound consists of an initial; rough dose range finding experiment, a subsequent experiment to narrow the range of effective doses for measurement of leathality and finally definative experiment for establishing a dose response curve for lethality.

When suitably extensive observations are made of the symptomatology of animals used for acute toxicity test, it is possible to estimate the minimal symptomatic or toxic dose, the maximal tolerated dose whereby the animals recover completely from all effect of chemical and the dose that produces no effect in the test species. On the basis of such information, estimations of the duration of action of single dose may be made to obtain information that can be used for subsequent prolonged toxicity studies.

PROLONGED TOXICITY TESTS :

The objective of prolonged toxicity test is generally to evaluate and characterize all effects of compounds when they are administered to the experimental animals repeatedly, usually on daily or alternate day basis over a period of 2-3 months. When the chemical under investigation is drug, the pharmacologic effects are particularly evaluated. When food addetives are under investigation; a prolonged test is usually followed by chronic or special types of test, therefore, the prolonged test supplies additional information which can be utilized in designing a long terms chronic test, as the duration of the toxicological test increases from single administration type of the test to

multiple repeated dose type test; two practical factors encountered, that limit the designing of the experiment and type of animal used. The first is that the available routes of administrations are limited because the route which is used must be suitable so that repeated administration of chemical compound does not include harmful effects in the animals. Second factor is that the properly designed prolonged experiment involves the use of the species of animals from which blood and urine sample can be obtained at intervals for clinical chemistry without inducing significant harm to experimental animals. Prolonged repeated test involve the application of analytical techniques for determining effects on blood chemistry and blood cells or urine chemistry and specific organ function. This includes hematology; blood chemistry; urine analysis and special function test. Prolonged toxicity test also involves the evaluation of animal for gross pathalogic and histologic effect at least at the end of experiment. Also, during the experiment any animal that become ill or moriband are usually sacrificed and a complete autopsy is performed. Clinical hematology, blood chemistry and urine analysis are performed at least at four weeks intervals and just prior to the termination of the experiment. At the termination of the experiment all animals are sacrificed and subjected to complete pathological examination.

One of the objectives of prolonged toxicity test is to attempt to demonstrate some form of toxic effect at least in the high dose group. Such effects may occur early in the

experiment or may not occur in the duration of experiment. If severe toxicity occur early in the experiment, that indicates selection of dose schedule was in error. Therefore, the animals are continued in the experiment at a lower dose schedule. In this manner the reversability of the toxic effect can be evaluated and experiment can be continued using a more realistic, maximal tolerated dose.

CHRONIC TOXICITY TESTS :

The basis of chemical induced toxicity proposed is, that toxicity is directly related to the concentration of the chemical at the effector site. Therefore, a sufficiently small concentration of any chemical should be compatible with the biologic system for indefinite periods of time. The thesis that, if a given concentration of chemical produces toxicity in 1 month, then one-half of that given concentration of the chemical would produce the same toxicity in 2 months is not tenable. Consequently chemicals that are to be administered to humans over periods of months or years should be tested in experimental animals over atleast comparable periods of time, by comparable routes of administration and by comparable doses as well as by excessive doses.

By the time a new chemical is considered for chronic toxicity studies, information has been obtained regarding the nature of its toxicity and its tolerable as well as lethal repeated doses. Also in the case of new drugs by this time, it should be feasible to give a few doses of the chemical to selected

human subjects under controlled experimental conditions in order to obtain enough confidence to indicate that the absorption; metabolic disposition and duration of actions of the compounds are similar between the human and the species selected for the chronic toxicity studies. Except for its academic value, there is little rationale for performing 1 to 2 year chronic studies on a species of animal that grossly differes from the human in its ability to absorb, distribute, metabolize or excrete the compound. If one species of animal is found to fulfill the requirements for approximating the human species, chronic toxicity studies on a species would be more meaningful. Because of the time, effort, and expense involved in conducting chronic toxicity studies in animals, any preliminary effort that is expended in determining the most suitable species for the test is well spent.

The progression of acute, sub-acute and chronic studies outlined above, is part of the requirement for testing prior to the administration of the new drug or product to the animal body. The basic requirements are as follows :-

(1) Chemistry :- 1) The constitutions, composition and identification of impurities, if any, the stability of the agent under various conditions, and other pertinent information must be obtained. ii) Relevent information must be known about the physico-chemical characteristics of the material such as its solubility in body fluids, affinity for proteins, dissociations characteristics. iii) Methods must be developed for the detection and identification of the substance of levels below those likely

to be present in sources of exposure or in body fluids.

(2) Biology :- i) The toxicity must be evaluated. Although subject to change for particular substances and classes, the phases of toxicity testing require the use of various routes in two or three species for varying length of time. The acute, sub-acute and chronic toxicity must be measured accompanied by detailed observation of physiologic, chemical and biochemical effects and pathologic changes. The studies often must be accompanied by the development or identification of suitable antidotes. ii) The kinetics of absorption, distribution, metabolism and excretion in the intact animal, the action on organs, tissues and cellular preparations, the biochemical interactions and to the degree possible, the mechanism of action must be ascertained, iii) Special studies may be required such as tests for sensitization, studies of reproductive function through three generations, the effect of nutritional factors, disease states, pregnancy, and for drugs, clinical trials.

The anticipated levels and mode of use of the agent must be established as well as the patterns of exposure likely to occur and blood levels expected. Factors that can modify toxicity must also be investigated. Some of the factors are related to the agent such as the type of vehicle, presence of adjuvants, and the physical form of the agent. Other factors are related to exposure characteristics, e.g. the dose and concentration and route of administration. Finally there are those factors that relate to the subject such as species, strain or race, age, sex, nutritional, genetic, hormonal, immunologic

status of the subject, existance of disease state, and environmental factors including chemical, physical and social components.

ROUTE OF ADMINISTRATION :

Toxic agents usually enter the blood stream of man after absorption from skin, lungs or gastrointestinal tract. However, in studying chemical agents, toxicologist frequently administer these chemicals to laboratory animals by various special routes, the most common of which are 1) intraperitoneal, 2) subcutaneous, 3) intramuscular, 4) intravenous. The intravenous route of administration introduces the toxicant directly into the blood-stream and thus the process of absorption is eliminated. The intraperitoneal route of administration of toxicant to laboratory animal is also common procedure. This method results in a rapid absorption of toxicant due to rich blood supply to the peritoneal cavity and to the large surface area. Compounds administered intraperitoneally are absorbed primarily through the portal circulation and, therefore, must pass through the liver before reaching other organs (Lukas et al., 1971).

Toxicant administered subcutaneously and intramuscularly are usually absorbed at a slower rate. The rate of absorption by these two routes can be altered by changing the blood flow to the area and by altering the solution in which the toxicant is administered.

The toxicity of a chemical may or may not be dependent on the route of parentral administration. If a toxicant is

injected intraperitoneally, most of drug will enter the liver via the portal circulation before it reaches the general circulation of animal. Therefore, an intraperitoneally administered drug might be completely metabolized or extracted by the liver and excreted into the bile and never gain access to the remainder of the animal. Any toxicant handled in such a manner that has a selective toxicity for an organ other than the liver and gastrointestinal tract would be expected to be much less toxic when administered intraperitoneally than when injected subcutaneously or intramuscularly.

II. URANIUM IN TOXICOLOGY :

Uranium metal is inter-transitional element showing properties of rare earth metals. Uranium constitutes the actinides series of the III B group of highly radioactive metals and the toxicity associated with the chemical nature of these metals is not fully assessed; due to their intense radioactivity; their deposition and transient retention in the bone enhances their intense radiation damage on the skeleton.

Uranyl nitrate [$UO_2(NO_3)_2:6H_2O$] a low radiotoxic derivative of Uranium, Mole.weight 502:13 is a water soluble salt and is invariably studied in toxicology. It is used in glass and ceramic industries and is a good photographic intensifier. Uranyl nitrate cause toxic effect 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 of uranyl

ion to phosphate ligands and to the inhibition of glucose transfer (Luckey and Venugopal, 1977).

Uranyl nitrate is a well known nephrotoxin and is long been used to induce experimental nephritis in different animals. Uranium in the form of one of its oldest substance to be used to induce a renal injury either for purpose of obtaining acute and chronic renal changes comparable to those developing in man; or for the purpose of inducing such changes and attempting to correlate the histologic findings with renal functional response. In 1854 Leconte first used uranium as nephrotoxic agent. Much later than this Chittenden and Hutchinson (1886); and Chittenden and Lambert (1888) became interested in the action of Uranium; Wallace and Myers (1913-1914) investigated the ability of the compound to induce a glycosuria. Schirokauer (1908), Heineke and Myerstein (1907) induced both severe vascular and epithelial injuries with it; which followed its use Christian (1908); Christian et al. (1911) and Christian and O'Hare (1913) described in detail the renal epithelial injury and also described the occurance of hyline droplets in the walls of glomerular capillaries. Pearce (1910) reported the uranium injuries restricted to the convoluted tubules. The selective affinity of uranyl nitrate for the tubular epithelium was reported by a series of research papers by MacNider (1912a)(1912b); (1913) (1914); where he pointed out that age of the animals determines in large measure the severity of the toxic response (MacNider, 1917). The reduction of alkali reserves of the blood in normal animals was also reported by the same author (MacNider 1918) and

that a protection against it could be established by the use of weak alkaline solution or glucose (MacNider, 1916; 1926). The injury in such animals was largely confined to a greater or less extent the histologic structure of these cells. These observations were confirmed by Goto (1917). The specific localizations of the nephrotoxic action of uranium was later carried on by Suzuki (1912) and Mitamura (1924).

In the same era Dickson (1912) reported his original work on chronic nephritis by the administration of uranyl nitrate. A considerable amount of work by Schlayer and Hedinger (1907) and Georgopulous (1906) focused the attention of investigators to the study of uranium nephritis, especially in the acute stages and to the related condition of edema formation. These studies have cast much light not only upon the effect of the renal lesion on the function of the kidney; but have also suggested a possible explaination for the progression of acute uranium lesions in chronic nephritis. The hypothesis advanced by Dickson (1912) that, there was a stimulative irritation of the connective tissue elements coincident with the destruction of the epithelium, was supported by the progressive nature of the connective tissue proliferation and its development around blood vessels, which showed uranium to be a vascular as well as an epithelial toxin. In this regard Ophulüs (1907) advanced his hypothesis; that if any toxicant acts simultaneously on both the connective tissue as well as epithelial cell lining, the cumulative degeneration is first noticed in epithelial cells in very short time than the vascular tissue. However in the view of Oliver (1915), the

marked degenerative effect of uranium nitrate administration in the epithelium and in the connective tissue were co-ordinate; not subordinate in nature. On the contrary, Aschoff (1912) and Suzuki (1912) showed that when uranium is injected in moderate doses it affects mainly the terminal part of the proximal convoluted tubule. In chronic uranium nephritis, which they found regularly after the acute lesion; the connective tissue proliferation starts in that region where the epithelial damage was greatest i.e. in the outer stripe of the outer zone of the medulla, for there the terminal division of the proximal convoluted tubules lie (Peter, 1907).

Experimental investigations in this regard have been largely confined to the regeneration following mechanical insult (Podwyssozki, 1887; Ribbert, 1904; Hochhaus, 1898) however, the results of such a gross attack on renal elements can be applied to a limited extent to the regeneration following the production of lesions by toxic agents, which reach to the blood stream. The only extensive study of regeneration following the administration of renal poisons have been made by Thorel (1903).

The experimentation on uranium poisoning was again accelerated by MacNider (1924, 1926) with his indifatigable interest in acute and chronic nephritis; attempted to correlate the histologic changes in the kidney with certain functional expression in the blood and urine. Mackay and Mackay (1930) have reported the resistance to morphine in the experimental uremia caused by uranium nitrate. The histopathological observation in this regard were reported by Hunter and Roberts (1932); where the

histopathological changes were confined to the glomerular region and the epithelial lining on the convoluted tubule in rabbits and monkeys, in both acute and chronic nephrotic models induced by uranyl nitrate.

In addition to the studies of uranyl nitrate as a nephrotoxin; many investigators have also studied the effect of this toxin at different tissue levels. Bobey et al. (1943) studied the plasma diodrast clearance and renal plasma flow in the dog. In the year 1945, Tripodo studied the enzyme tributyrin lipase in blood after the intoxication of uranyl nitrate in rabbits, where he observed a gradual progressive increase in the lipolytic power of plasma. Laake (1945) reported changes in excretary as well as reabsorptive patterns of different constituents after the uranyl nitrate administration. Aldo Mastarzo (1950) conducted the experiments in Guinea pigs and Rabbits, and reported the normochromic anemia with a tendency toward hyperchromia in the blood and hyperplasia in bones. A change in serum lipid metabolism was reported in rabbit with uranyl nitrate poisoning by Bauer et al. (1951). He reported a consequent azotemia and lipemia after the uranyl nitrate administration. Schulze et al. (1955) also reported a change in lipid metabolism of dog after the administration of uranyl nitrate and increase in the neutral lipid content of the experimental animal was remarkably observed.

The Manhutten project sponsered by USA government have reported a extensive work on the poisoning of uranium and its derivatives. Barnett et al. (1949) from this project has studied the pathological anatomy of uranium poisoning and reported

necrosis of renal tubular epithelium involving principally the proximal convoluted tubules of rats; dogs; rabbits; guinea pigs and mice. Even a low dose of uranium causes damage in the junction of the middle and distal third of the proximal convoluted segments; which was linear to the dose concentration. The peak of necrosis was attained in 3-4 days of the treatment in higher doses. In addition to renal injury the degeneration was also observed in liver cords; and mucosal and submucosal hemorrhages in the gastrointestinal tract. Orcuff et al. (1949) from the same project; pointed out the effect of uranium on eye of the rabbits; in low as well as high doses of uranyl nitrate; it produced sever damage; edema; corrosion; vascularization and corneal loadiness in eye region. Haven et al. (1949) working in the same project; studied the tolerance of the animals to uranium compounds; according to them, male rats possessed more natural and developed greater tolerance than females. Increased ratio of kidney to body weight decrease in percent of dry residue, absence of kidney phosphatase; high urinary volumes and low variable pH; all indicated the kidney damage in the tolerant rats. The apparent LD_{50} values reported for uranyl nitrate were 10-20 mg/kg in mice; 20-25 mg/kg in C_3H mice; 0.1 mg/kg in rabbit; 0.3 mg/kg in guinea pigs and 1 mg/kg in rat.

Dounce et al. (1949) has published a review article on mechanism of action of uranium compounds in the animal body to furnish explainations for the mode of primary action of uranium compounds on the body. He has reported a local damage of eye, lung and to a slight extent of the skin, by exposure to uranium

compounds. The readily soluble uranium compounds like uranyl nitrate; uranyl acetate, UCl_4 and UCl_5 hydrolyse to produce acid. Even 0.1 M uranyl nitrate in aqueous solution has pH of 4.0 or below. Serious lung damage might be produced by HCl resulting from hydrolysis of uranyl nitrate particle lodging there. After the entry in the blood uranium combines with blood proteins, especially the albumin and is not diffusible in that state, whereas uranium complexing with HCO_3 (bicarbonate) in blood, is diffusible (Barron, 1951; Tannenbaum and Silverstone, 1951). The distribution study of uranyl nitrate after its entry into the animal body has been done on rats following large intravenous doses of uranyl nitrate (3-5 mg/kg body wt). It has been found that the two chief sites of uranium deposition are the bone and kidneys. However, uranium in kidney decreases fairly rapidly, whereas the uranium in bone decreases very slowly indicating that bone is the most important site of storage in the body. Similar results were obtained by Neuman et al. (1948) and Tannenbaum and Silverstone (1951). In kidney, the cortex is the essential site of accumulation with some tendancy of concentration at the corticomedullary junction. The distribution of uranium at blood level showed that the blood level falls rapidly, so that after 1 hr. over 95 percent of the uranium has left the stream and gone into bone or kidney.

The autoradiographic studies carried out by Wills and Neuman (1949), showed that the radioactive uranium was concentrated in certain areas of kidney more than in others and that in particular; some nephrones appeared to have concentration of

uranium compared to others. Although most glomeruli did not show much uranium. Occasional glomeruli appeared to show heavy concentrations. The microstaining reactions for localizing uranium in kidney carried out by Ander and Hutchens (1949) showed that uranium tends to be located in intensely staining rings or collars on the inside surface of some of the tubules, there was a rather generalized weak staining of some of the tubular epithelial cells and uranium was detected in the glomeruli.

The general route of excretion of uranyl nitrate in all animals so far studied (rats; rabbits, cat and dogs) appears to be by way of urine. Roughly uranium^{213} was excreted rapidly via urine (Neuman et al., 1948; Tannenbaum and Silverstone, 1951). During the same era many of the investigators have produced, literature confined to more profound study regarding the distribution, accumulation and excretion of uranium in the animal body (Meyer et al., 1951; Ferriti and Schwarbe, 1951; Barron, 1951; Muntz et al., 1951). Few interesting results have also been reported pertaining to the age factor, which were neglected earlier. MacNider (1946) and Maynard (1953) have reported that age factor has something to do with the suceptability, where older animals were proved to be more susceptible; where as young ones had greater tolerance.

III. EFFECT OF URANYL NITRATE ON BLOOD :

During past few decades extensive studies have been done on uranium poisoning in variety of animals (Nomiyama and Foulkes,

1968; Brown et al., 1970; Flamenbaum et al., 1976; Stein and Soprkin, 1976; Flamenbaum, 1977). However, there are very few reports which are related to the biochemistry and hematology. The effect of UN (Uranyl nitrate) on serum lipid metabolism have been extensively documented (Bing et al., 1925; Politzer, 1936; Popjack, 1945; Haymann et al., 1945; Bell, 1947; Gojer and Sawant, 1985).

Bauer et al. (1951) have reported lipemia and azotemia following administration of UN in rabbit. They have reported a elevation in serum lipid fraction, where all lipids participated in this increase, but the greatest increase was in the neutral lipid fraction. Prior to this study, Tripodo (1945) has reported a gradual progressive increase in lipolytic power of plasma under UN intoxication, which reached its maximum between I & II week of the treatment. Dounce et al. (1949) have also reported a UN induced change in phospholipids and cholesterol metabolism, where the unsaturated fatty acids were observed to be increased in blood of UN induced rat. On the other hand chloesterol level in the blood was found to be decreased in UN poisoned rats. In the recent years many reports on ARF (acute renal failure) have been published (Bencosme et al., 1960; Popvitzer, 1979; Avasthi et al., 1980; Yamaguchi, 1980; Yano, 1982).

The above reports have cast light upon the effect of UN on renal dysfunction (ARF) and metabolism under the influence of UN toxicity, but no studies have been done to gain an insight into the effect of UN on blood corpuscules and hematological

values to understand the early pathological indications of ARF, which may have a clinical dignostic importance. There are few reports pertaining to the hematological alterations during UN induced ARF. References have already been made to certain intraerythrocytary enzyme changes in hemolytic conditions induced by UN (Fedorova et al., 1966). Meola et al. (1982) has devoted his work to the effect of UN on the derangement of red blood cells exposed to a sufficiently higher concentration (5 to 10 mg/kg) of UN showed cytoplasmic bridges between the discoid cells. As the time interval increases (96 hrs.) the bridges become clearly visible. According to him the cytoplasmic bridges are indicative of a severity of ARF induced by UN and if presence of cytoplasmic bridges between erythrocytes can be correlated with early renal dysfunction would be very useful and dignostic tool. Our laboratory in this regard has done a considerable work on hematology of mice in the various phases of ARF induced by UN (Gojer & Sawant, 1985) which inspired to take up the present investigation.

IV. WORK ON URANYL NITRATE TOXICITY FROM THIS LABORATORY :

The Laboratory of Animal Physiology, Zoology Department, Shivaji University, Kolhapur has been actively engaged in extensive work on UN toxicity and UN induced ARF. In the initial stage, Sagre and Sawant (1976) worked out that, after intramuscular injection of UN (6 mg/kg, body wt.) there was change in the lipid patterns of male white mice kidney. Increase in the total, neutral and phospholipid fraction continued to the peak period of

72 hours and then followed by a sharp decrease of normal values at 76 hours. Quantitatively alterations in the individual components of neutral and phospholipid resembled total lipids, but the extent of increase and earlier work of Goyer & Rhyne (1973) and Schulze et al. (1955); concluded that there is persistent state of lipemia produced after UN administration. Considering above view in mind, it was planned to work out the lipolytic enzyme that are involved in the impaired lipid metabolism under the toxic influence of UN.

Gojer and Sawant (1985) worked out the basic toxicologic studies in rat and mice, which include study of physical and behavioural observations after UN administration, effect of sex and age on LD_{50} value and a possibility of aquisition of tolerance in case of animals administered with UN. In the same year they worked out the effect of UN at two different dose concentration (10 mg/kg and 25 mg/kg) on the adipose tissue of mice, where a detailed comprehensive data on purification and isolation of tri acyl glycerol hydrolase in mice adipose tissue has already been published (Gojer and Sawant, 1985). The effect of UN on lipolytic activity of mice adipose tissue from different anatomical sides has also been worked out, since adipose tissue has a crucial role in biosynthesis and degradation of lipid content, it is reported that there is persistant elevation in the tri-acyl-glycerol hydrolase activity of mice adipose tissue throughout the different phases of ARF and the consequences of which is, lipemia (Gojer and Sawant, 1985a). To probe a chance of such disturbances in lipid metabolism of serum; Gojer et al. (1985) studied the mice

serum under the toxic stress of UN which again showed a persistent elevation in the tri-acyl-glycerol hydrolase activity throughout the different phases of ARF.

UN being a nephrotoxin, its effect on renal lipolytic activity was thought obvious and Desai and Sawant (1984) reported a lipolytic disturbances induced by UN. A pertinent interpretation of which has also been reported, considering the vital organ systems like brain, liver, kidney and the serum. Some preliminary observations on rat hematology after UN administration and its clinical importance has been reported by Patil *et al*. (1986) this report forms a part of the present thesis. Recently Gojer and Sawant (1986b) have suggested use of sodium loading and a antidote named, dithiothreitol in the preventive and curative therapy of UN induced ARF. The biochemical, histopathological and hematological work in this regard has also been reported extensively. Most of the above mentioned work is either discussed in various national symposia or is published in standard international journals. The advanced work in this regard is in progress.

V. REASONS THAT LED TO THE PRESENT INVESTIGATION :

Metal toxicity have long been considered as an occupational hazard due to wide industralization in the recent years. In the past few decades man and animal are exposed to a wide array of metals that would otherwise have stayed burried or localized. In order to survive in a developing technologic age, it becomes indispensable to understand the untoward effects of different concentrations of metals and its compounds on human beings,

which could impart a better idea for rational utilization of metal compound in our lives. Increasing efforts have been extended to the problem of metal toxicity and safety in man and animals in the recent days.

The increasing use of rare earth compounds in different fields has stimulated the interest of studying toxicity of these metals. Uranyl nitrate, one of the rare earth metal widely used in industries is known to affect the morphology and ultrastructure of several vital organs. It has become a target of several investigators due to its marked effect on renal morphology and physiology (Tannenbaum and Silverston, 1951; Bencosme et al. 1960; Flamenbum, 1973). Effect of uranyl nitrate on blood chemistry has also been well documented (Tripoda, 1945; Bauer et al., 1951; Schulze , et al., 1955). In recent years use of uranyl nitrate as a nephrotoxin to induce experimental acute renal failure (ARF) has uplifted the interest of many scientists who were keen in understanding the basic pathophysiology of ARF? Although extensive work has been reported about the toxic effect of uranyl nitrate; its mechanism of action, biochemical and ultrastructural consequences during recent years, very little is known about its effect on hematology of the man or animal. There are very few reports available on man affected by uranium derivatives (Polski, 1963; Meola, 1982). But, these studies do not offer a detailed comphrehensive data or pertinent interpretation to direct us to a desicive concept in dignostic therapy. Hence the hematological studies after uranyl nitrate treatment has remained hitherto neglected area in this field.

Red blood cells circulating in peripheral blood, share in part the structural and functional features common to most cells, but lacked others. Biochemically, the cell interior possess the metabolic apparatus for providing energy, the synthetic machinary for manufacture of micromolecular bioconstituents is absent. Neither is there the presence of compounds of mitochondrial respiratory chain involved in biological oxidation process. While maintenance of the normal red cell constituents, clearly, is of crucial importance for the internal function within the red cells. Preservation of these is dependent on the intactness of the erythrocyte membrane. It is this very membrane which is obviously a major target for interaction with toxic factors. Generally, the membrane of matured red cell is quite vulnerable to many physico-chemical influences. Indices of which are useful in the detection of red cell damage brought about by exogenous causes are diverse and include (1) Red cell morphology, (2) Cell viability, (3) Integrity of membrane, osmotic resistance (mechanical fragility),(4) Functional properties of membrane and (5) Intra erythrocytory functions. Structural and functional defects of red cells do arise as a result of in vivo treatment with vast array of chemical and physical agents. Such defects may eventually terminate in development of anemias of various kinds.

Lytic attack on red cells, eventually ending in hemolytic anemia, unmistakably follows the massive absorption of variety of industrial chemical and drugs. Furthermore, hemolysis may be produced by certain nutrients; plant constituents and snake venom. In their detrimental action, such hemolytic poisons

produce lysis of red cells, with consequent loss of intracellular hemoglobin. Hemoglobin thus released into blood, plasma is subject to increase distruction of its heme portion by breakdown into bile pigments (Truhaut & Bohuon, 1963; Prelli et al., 1963, 1970, and Tarlov, 1962).

Chemicals which are found to possess hemolytic action include arsenic, alkyl chlorates, methyl chloride, phenyl hydrazine, p-dichlorobenzen, chloronitrobenzen, p-phenyl diamine, napthalene, nitrozomine, toludene, toludene diamine, tri alkyl; tri aryltin salts and selenites, heavy metals-copper, cadmium, lead and nephrotoxins also exhibit hemolytic potential to different extents (Gojer and Sawant, 1985). Lysis of red cells caused by these agents involves a multiplicity of mechanisms but is invariably associated with a primary fall in the intracellular level of reduced glutothione to values incompatible with the life of cells (Feglar, 1952; Pernis and Magestretti, 1960). Diminished viabality of red cells in response to toxic insult is usually expressed by a shortened cell survival time, and alteration in functional characteristics of the red cell membrane, references have already been made to certain intraerythrocytary enzyme changes in hemolytic condition under the influence of uranyl compound (Federova, 1966).

With a critical view of extensive data available on uranyl nitrate and heavy metal toxicity, vulnerability of blood corpuscules under the influence of toxic agents and the preliminary hematological observations carried out in our laboratory bring about some interesting aspects in hematology of rat under

the toxic influence of uranyl nitrate which are either not worked out or very little is known about them.

(1) Since blood is first and foremost media that is exposed to the toxin, it is essential to study the early effect of toxin at blood corpuscular level. The data on which may provide us better clues on its mechanism of action.

(2) The physical and observational examinations of rat and mice under the experimental intoxication with UN has shown that, 48 hours after the administration of UN, animal looses its appetite and obviously its weight. As a result animal gives anemic look (Gojer and Sawant, 1985), whether this conditions is reflected in early hematological studies is interesting.

(3) A comparative data of lead, cadmium, aluminium toxicity on hematology is available (Goyer and Rhyne, 1973). However, no studies have been addressed to the hematologic alterations induced by uranyl nitrate, which may possibly have a dignostic importance.

(4) Meola et al. (1982)has reported a corpuscular derangement, 48 hours after UN administrations, Study of red blood cells at various magnifications is warranted for a better understanding of pathophysiologic mechanism of ARF.

(5) There is contraversy regarding the mechanism of action of UN on the cell membrane. It will be interesting to study the effect of UN on integrity and permeability of red blood cells which could give a better explanation for mechanism of action of UN.

(6) Heavy metals like lead, cadmium, mercury and copper are known

to cause hemolytic anemia forming ligands with erythrocytes lipoprotein membrane. Wheather UN causes anemia due to hemolytic interaction forming ligands with erythrocytes? or impaired erythropoiesis? The etiology of UN induced anemia deserves further investigation.

(7) Since UN is a known nephrotoxin, there is every possibility that it will affect the synthesis of renal erythropoetin, crucial in the genesis of erythrocytes. To ascertain such possibility, study of R.B.C. count at the various phases of UN induced ARF is inevitable.

(8) Another possibility of erythrocyte degeneration at bone marrow level should equally be considered. Since UN is known to accumulate in bone, the possibility of such effect on R.B.C. production should be probed.

(9) Hypocoagulation is common finding in majority of experimental metal toxicity e.g. lead, aluminium, chromium, mercury, silver, cadmium. The effect of UN on coagulation of blood is interesting and hence desired.

(10) The elevation in tri-acyl-glycerol hydrolase activity in serum and blood urea nitrogen are the parameters that indicate the severity of UN induced ARF in the experimental animal (Gojer and Sawant, 1985). The relationship between hematological alterations and the biochemical alterations in the lipolytic activity of serum and blood urea nitrogen is hence felt desirable.

VI. PLAN OF PROPOSED WORK :

Keeping in mind all above aspects which indicate the importance of the studies on hematological alterations manifested during UN induced ARF, it was proposed to take a detailed investigation of the effect of UN at blood corpuscular level including other important hematological values and the biochemical alterations in lipolytic activity and blood urea nitrogen during the different phases of UN induced ARF.

The plan of proposed work thus consists of -

(1) To obtain the basic toxicological information about the UN in rat, such as selection of dose concentration, dose interval, route of administration and determination of LD_{50} value etc.

(2) To determine the effect of UN induced ARF on rate of coagulation in different phases of ARF at two different dose concentrations.

(3) To determine the effect of a sublethal and lethal dose of UN on fragility of red blood corpuscles.

(4) To study the effect of UN at two different concentrations on erythrocyte sedimentation rate in the different phases of ARF.

(5) To study the effect of UN (5 mg/kg and 10 mg/kg body wt.) on the hemoglobin concentration of UN administered rat in various phases of ARF.

(6) To determine the effect of UN induced ARF on total R.B.C., W.B.C. and the differential count of the UN administered animal.

(7) To calculate the hemotocrit values of the animal under toxic influence of UN (5 mg/kg and 10 mg/kg body wt.). This include determination of mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration in UN administered animal.

(8) To develop a sensitive assay of tri-acyl-glycerol hydrolase in rat and study the behaviour of tri-acyl-hydrolase activity in rat serum under the influence of UN induced ARF.

(9) To estimate the blood urea nitrogen value both in normal and UN administered rat to study the pathogenic condition of animal under the toxic stress of UN.

(10) The above mentioned study comprises two different tests I) acute toxicity test and II) prolonged toxicity test. All the parameters mentioned above were studied separately for above mentioned tests. In light of the various suggestions made with respect to biochemical changes in blood chemistry and the hematological picture under UN induced ARF. To project an idea regarding the physiological and clinical significance of hematological alterations during UN induced ARF in the experimental animals.

For the present investigation standard and accepted hematological and biochemical techniques have been employed. Wherever needed the possible modifications were made in the available techniques to get correct and authentic data on hematological and biochemical events that intervene during UN induced ARF.