CHAPTER - I

INTRODUCTION :

Review of literature, reasons that led to the undertaking of the present investigation and plan of Proposed work.

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"For the Life of all Flesh, is the blood thereof."
- Leviticus, 17; 14

Life evolved first in sea water. The primitive unicellular organism took it's oxygen and nutrition from the ocean and excreted it's waste products into it. With development of the multicellular forms, this simple arrangement could not hold viable, as the cells in deeper parts of the organisms could not come in contact with the surrounding medium. To overcome this, a system of intercommunicating channels was developed. Through these channels the sea water could freely flow in and out and thus the deeper cells could fulfill their needs. Such a system represents the beginning of an open circulatory system. With the evolution of higher animals the open vascular system turned into a closed one. This change represents an important landmark in the history of animal evolution. The Sea water, which used to remain outside the body, became entrapped within the body and profound modifications took place and in due course of time it was transformed into blood.

Blood is a highly complex fluid, which is composed of two parts- Plasma and formed elementary particles. The plasma is composed of plasma proteins and other organic and inorganic components. The plasma proteins are considered together as a system of proteins because of their similar biosynthetic origin, their participation in common processes and their occurrence together as the major extracellular components of the circulatory system.

The protein system is a dynamic one and has various functions. The primary functions are the maintenance of colloid

osmotic prəssure, pH, and electrolyte balance, the transport of metal, ions, fatty acids, steroids, hormones, drugs etc. They readily provide the amino acids for the tissues. Hemostasis and the prevention of thrombosis is with the help of plasma proteins. They regulate the cellular activity and function via hormones, and take defencive action against pathogens with the help of antibodies and other plasma factors. In disease, plasma proteins fluctuate in their normal values. This reason created an early interest in the plasma proteins and it led to innumerable clinical studies and searching investigaticns of the nature and functions of plasma proteins.

The plasma protein vary greatly and thus, create a problem for the identification, classification and nomenclature. A comparative study of plasma protein biochemistry is a new approach to the developmental biology and phylogenetic relationships between the organisms (Engle and Woods, 1960). The relation between composition, structure and functions of plasma proteins has been studied by many investigators.

New systems are evolved for grouping of plasma proteins with the introduction of free boundry electrophoresis by Tiselius (1937). Each grouping, system and techniques for separation led to new and conflicting classification. Thus, five basic problems, viz. the resolution, identification, fractionation, purification and characterization have to be considered in connection with plasma proteins. The resolution depends on a separation based on differences

in the solubility under defined conditions, net electrical charge, rate of ultracentrifugal sedimentation, immunological specificity, or a combination of these. Thus, the recent techniques of starch gel electrophoresis, immunoelectrophoresis, and chromatography have facilitated a better resolution and identification of the plasma proteins. In this regard the contribution of Schultz and co-workers (1955,1962) must be appreciated.

Grabar (1963), has pointed out many difficulties in the nomenclature, which are compounded. When more than one functional name is given to a single protein, for example the presently named transferrin was used to be called β_1 iron-binding globulin (Surgenor et al., 1949) and siderophilin (Schade et al., 1949). Now the goal in plasma protein nomenclature, is to eliminate the designations based on the method of detection or the fractionation procedures and to assign new names denoting the special function of any protein, once it has been isolated and characterized.

The diversity of the plasma proteins is obscured by consideration of them as a single extracellular protein system. The high molecular weight of the serum proteins is the main reason that they have so long resisted structural study, and very little is known about their primary structure.

The characteristic of proteins, to aggregate, is of much importance. Albumin readily forms dimers and all the three classes

of immunoglobulins readily form poymers that can be dissociated by sulfhydryl reagents. Thus, one of the greatest sources of difficulty in the measurement of the molecular weight of some plasma proteins is the occurrence of aggregation, often as the result of denaturation during isolation and storage.

The most characteristic non-peptide moiety in plasma proteins is carbohydrate. Carbohydrates - occur in virtually all the known plasma proteins, with the exception of albumin. Other common components of plasma proteins, particularly in the α and β globulins, are the lipids. Among the remaining constituents are the metals, and the same have been overlooked a number of times (Laurell, 1960). Albumin, transferrin, haptoglobin and ceruloplasmin are mainly involved in the metal binding.

STRUCTURE AND FUNCTIONS OF PLASMA PROTEINS :

A) ALBUMIN :

In the Plasma Protein System, albumin (or plasma albumin, as it is sometimes called) occupies a paramount position. The ready availability of crystalline albumin has made it one of the most widely investigated proteins. Numerous studies have been made on the ion-binding behaviour of this protein. Its amphoteric properties, its conformational changes and electrophoretic heterogenity at low pH and its denaturation characteristics have been studied. The literature on these aspects is so extensive that it cannot be adequately covered

here. The review of Foster(1960) is important both for its summary and its interpretation of the data.

Albumin is often regarded as a single homogeneous protein with similar physicochemical properties in all species for which the crystalline protein has been isolated. However, exceptions to this statement have often been noted. Albumin readily aggregates at low pH and it rapidly forms a dimer in the presence of Mercury or organic Mercurials. It exhibits heterogensity upon free electrophoresis at the isoelectric point and at more acid pH.

Albumin is considered to be similar in all the vertabrate species, though undoubtedly differing in its primary structure. One evidence, for this, is the similarity in molecular weight indicated for several species. The molecular weight generally cited for human albumin is 69,000 D., although some authors prefer the figure of 65,000 (Low, 1952; Hughes, 1954; Charlwood, 1961; Scatchard and Pigliacampi, 1962). The apparent variation in values for different species is probably attributable to the inherent error of the methods, the presence of contaminating globulins and the bias introduced by the usual presence of dimers and higher aggregates. Charlwood (1961), after careful measurements, reported a value close to 65,000 for human, rat, rabbit and guinea pig albumins.

Since the observations of Luetscher (1939) and Sharp et al (1942), the heterogeneity of albumin in moving boundary

electrophoresis near pH 4 has been the subject of recurrent investigation. Albumins of various species, migrate with a single moving boundary at pH above the isoelectric point(pH 4.7) but yield two and sometimes three boundaries between pH 3.5 and 4.5. The patterns usually are not enantiographic and are greatly influenced by the ionic strength and the buffer composition. This behaviour has been attributed by Phelps and Cann (1956) and by Cann (1960) to the interaction with acetate ions; but has been interpreted by Aoki and Foster (1957) and Foster and Aoki (1958) in terms of a cooperative alteration in the portein conformation, i.e. an "Isomerization", which takes place near pH 4.

The unusually strong affinity of albumin for anions and other substances has made it a model for study of protein-ion interaction. (Klotz (1952) reviewed this aspect with particular reference to albumin). The most remarkable example of this is the interaction of albumin with detergent ions such as dodecyl sulfate. This phenomenon, first reported by Putnam and Neurath (1944), has been studied extensively by many workers and is reviewed by Foster (1960). Various models to explain this remarkable affinity rely on the configurational changes that albumin so readily undergoes.

The physiological consequences of the ion-binding behaviour of albumin and its affinity for dyes, drugs, and other molecules have been emphasized by Bennhold (1962). This phenomenon has considerable pharmacological significance.

B) TRANSFERRIN :

The major part of acid soluble iron in plasma is reversibly bound to a specific metal - combining protein named transferrin for its function of transporting iron to bone marrow and tissue storage organs. The physiological significance of this protein is apparent from its central role in the cyclic process whereby iron derived from the catabolism of hemoglobin and other proteins is conserved by its almost quantitative return to hematcpoietic tissue. Transferrin also participates directly in the regulation and control of iron absorption and protects against iron intoxication. The existence of a plasma iron-binding protein was suspected for some time but first demonstrated by Holmberg and Laurell(1945) and independently by Schade and Caroline (1946). Holmberg and Laurell showed that serum iron does not react with the complexing agent α,α' - dipyridyl until a saturation limit of about 315 μg iron/100 ml is reached. Since, transferrin normally is only about 30% saturated, plasma changes from yellow tc yellowred on the addition of ferrous iron. Schade and Caroline (1946), have indicated that the iron-binding protein was present in ethanol fraction IV. Subsequently, it was isolated and crystallized with the ethanol fractionation method by Sargenor et al. (1949) and by Koechlin (1952), who called it the β_1-metal-combining globulin. As early as 1952, Laurell, noted in his review that more than a thousand investigations had been made on the physiological and clinical aspects of plasma iron transport. Smithies subsequent discovery in 1957 of heritable transferring variants has stimulated

many investigations on the distribution and structure of these polymorphic proteins. Major reviews on the properties of plasma transferrins have been written by Schultze et al. (1956) and Laurell (1960) and on the genetic polymorphism by Giblett (1962) and Barnicot (1961).

Transferrin has a solubility more like that of albumin. It is a glycoprotein with a total carbohydrate content of 5.5%, distributed among hexose (2.4%), hexosamine (1.6%), and sialic acid (1.4%) (Schultze et al., 1957; Schultze, 1962). It has a molecular weight of about 90,000 based on sedimentation diffusion measurements, light scattering, osmotic pressure, and iron binding (Schultze et al., 1957).

Each transferrin molecule can combine with two atoms of ferric iron in an ionic bonding in which one bicarbonate ion is taken up per iron atom (Schade et al., 1949). The binding of iron is pH dependent. The complex is stable in the pH range from 7.5 to 10.0 but is dissociated on acidification to pH 4.0 and the iron can be removed by dialysis. Transferrin can also combine loosely with copper or zinc ions. Transferrin is colorless in the absence of iron and pink in its presence; it has an absorption maximum at 470 mμ and a minimum at 410 mμ. Spectropolarimetric study of human tranferrin and of albumin shows that the visible absorption bands of the iron complexes of these proteins are optically active. Two moles of iron are bound per mole of protein.

The carbohydrate portion of human transferrin contains sialic acid, galactose, mannose, and hexosamine in the molar ratio 4:8:4:8 (Schultze et al., 1958). Isolation of the glycopeptides has been undertaken by Jamieson (1963), who in a preliminary report suggests that four chains are present. Each would contain two hexosamines, two galactoses, and one mannose, with one molecule of sialic acid at a terminal position.

C) HAPTOGLOBINS :

The haptoglobins are a family of glycoproteins found in the α_2-globulin fraction of many mammalian species and were so named by Jayle and associates because of their ability to form specific stable complexes with hemoglobin (Polonovski and Jayle, 1939). Mol.Wt. 85,000 (with Hb combination - 3,10,000).

The biological function of haptoglobin is to bind hemoglobin strongly and thus, prevent undue loss of iron through urinary excretion. It also protects the kidney from damage by hemoglobin. It is the major factor regulating the renal threshold for hemoglobin, for as Laurell and Nyman showed in 1957, free hemoglobin cannot be detected in the urine unless the amount injected exceeds the haptoglobin-binding capacity. Haptoglobin is diminished in severe liver disease but is usually increased in inflammatory disease (Nyman, 1956). Thus, its determination has a clinical value.

D) CERULOPLASMIN :

A copper-binding protein with oxidase activity was isolated from serum in 1948 by Holmberg and Laurell, who named it ceruloplasmin on account of its blue color. Its purification, properties, function and biological variations have been reviewed by Laurell (1960). Ceruloplasmin is a glycoprotein present in the α_2-globulin fraction.

E) GAMMA - GLOBULINS :

The subject of γ-globulins is inextrically linked to that of antibodies because the primary and perhaps the sole function of γ - globulins is their immunological potential.

The pathological globulins are not themselves antibodies; indeed, the patients that produce them are deficient in their capacity for antibody production because the pathological globulins are formed by tumors of the very cells normally engaged in antibody synthesis, - the plasmocytes and lymphocytes. Thus, the study of the structure of abnormal globulin would provide a basis for structural study of antibodies.

The single most frustrating obstacle to establish a coherent model for the structure of γ-globulins of various species has been the inability to obtain consistent stoichiometric values for the N-terminal groups and thus, to ascertain the number of polypeptide

chains. That the γ -globulins of every species are heterogeneous in chemical structure. However, it may simply reflect on the limitations of the methods or the presence of acetylated amino end groups.

II) METAL TOXICITY :

Alterations in the protein metabolism is an important accompaniment of toxic renal and liver damage following the entry of a wide variety of heavy metals in the body. Depending on the causetive agent, the toxic action may be associated with various kinds of biochemical anomalies in the blood. Thus, the alterations in the compositicn of plasma proteins occur in various toxicologic states. Analysis of the relative distribution of the protein fractions will give valuable clinical information in nephrotoxic insult as well as in hepatotoxicity.

Increasing industrialization is creating a havoc in the natural balance. The industries release pollutants in the environment which cause adverse effects on plants, animals and also on inanimate things. The use of metals is common in the industries which produce weapons, instruments, herbicides, pesticides, etc. Photography, glass and ceramic industries also make use of metals. The waste of these industries when discarded without taking any precautions for avoiding pollution, causes the metals to get accumulated in recidual form and pollute food, water and air. Excess intake of these pollutants causes toxicity.

The symptoms of heavy metal toxicity in mammals are early mortality, growth retardation, impaired reproduction, depression of physiological parameters, neoplasm and chronic diseases. At cellular level membrane permiability and antimetabolic activities are the effects. Changes in enzyme inhibition and irreversible conformational changes in macromolecular structures are some of the effects at molecular levels by metal toxicity. The details of toxic effects of some heavy metals are as follows :

Copper in chronic poisoning causes nausea, vomitting, dizziness, jaundice, general debility. The acute poisoning symptoms are sporadic fever, hemolytic anemia with intravascular hemolysis, coma, cardiovascular collapse and death. Rat and pig can tolerate 200 ppm copper in their diet. Pigs show no sign of copper toxicity when fed diet containing 250 ppm copper and indicate growth stimulation when adequate amount of iron is added to the diet (Barber et al., 1955, 1956; Ritchie et al., 1963; Shuttle and Milli, 1966).

Sutton and Nelson (1937), studied the Zinc intoxication, causing anemia of the hyperchronic type, decreased erythrocyte production, formation of immature erythrocytes and increased leucocyte production. In earlier reports, Zinc was found at high concentrations in mammary tumors induced by other carcinogens (Tupper et al., 1955).

Acute poisoning of Cadmium leads to atrophy, hemorrhagic necrosis, vascular injury, increased permiability and decreased regional flow. According to Nicaud et al. (1942), bone disease is a diagnostic symptom of Cadmium exposure.

According to Kazantzis (1966), common symptoms of chronic Mercury poisoning are anoxia, unco-ordinated movements of arms and legs, impaired hearing, loss of teeth, impairement of taste and smell, while acute toxicity involves nausea, headache, abdominal pain and diarrhea, metalic taste in the mouth, albuminurea, hemolysis and death resulting from extreme exhaustion. Mercury accumulates in brain, liver, kidney and blood (Iwata et al., 1973).

Acute effects of Lead are lassitude, vomitting, loss of appetite, unco-ordinated body movements, convulsions and stupor eventually leading to coma and death. Chronic poisoning causes loss of appetite, vomitting, renal malfunction, hyperactivity, mild anemia, liver cirrhosis, brain damage and general intellectual and psychological impairement and reduced immunity.

A) **URANIUM TOXICITY** :

Despite the shortage of investment capital, concern for reactor safety and problems of radioactive waste etc., the nuclear industry has moved forward and considerable changes took place in the production technology of Uranium.

Uranium (U) is distributed more abundantly in the earth's surface than Bismoth, Cadmium, Silver, Mercury and Gold, averaging about 3 gm/ton of rock where it occurs. All U isotopes are radioactive. The U^{238} occurs naturally (99.25% of total). It has a half life of 4.51×10^{9} years and it is less radioactive than U^{235} and U^{233}. Uranium forms numerous compounds as well as cationic and anionic salts. Although there are more than hundred uranium bearing minerals, Carnotite ($K_2O.2U_2O_3.U_2O_5.3H_2O$), Pitchblend ($UO_3.UO_2$,PbO, Th, Y etc.), tobernite [$Cu(UO_3)_2P_2O_8.12H_2O$] and few others are of commercial importance.

Uranyl nitrate (UN) is a well known nephrotoxin and has long been used to induce experimental nephritis in different animals. Leconte (1954), first used Uranium as nephrotoxic agent and since then, it has been in the research field as a toxicant.

The reaction of alkali reserves of the blood and uranyl nitrate in normal animals was reported by McNider(1917b). Tripodo (1945) studied the enzyme tributyrin lipase in blood after the intoxication with UN in rabbits, where he observed a gradual increase in the lipolytic power of plasma.

Effect of Uranium (U) on rabbit renal tubule was studied by Bowman and Foulkes (1970). According to them the Lesions developed on the distal portion of the nephron. A decrease in the fresh water clearance occured during diuresis. Reduced capacity of water

conservation during hydropenia and dissipation of the normally high tissue sodium concentration in the renal medulla was also observed (Bowman and Foulkes, 1970).

The toxic action of U compounds during cutaneous application using a neutron activation method was studied by Arifov and Berent (1971). Application of U nitrate, U fluoride, U tetrachloride or U pentachloride to the skin of rats, mice and rabbits, increased the quantity of protein in the Urine. Neutron activation studies revealed U in blood after 4 to 6 minutes of it's application and finally it was accumulated in kidney, due to which the degradative changes were initiated. Further, implications include the refusal of food, weight loss and death of the treated animal.

Combined effects of uranium and radium on the whole body was observed by Svyatkina and Novikov(1975). In rats there was decrease in the erythrocyte, leucocyte, reticulocyte and lymphocyte counts. Changes in the activities of blood alkaline phosphotase and cholinesterase were also determined. The elements caused granular dystrophy of the renal tubule epithelium. Significant dystrophic changes were also observed in the gastrointestinal tract (Svyatkina and Novikov, 1975).

Fillipp̊va et al. (1978) studied the long term consequences following administration of enriched U to rats. They used U(IV) and U(V) and found, that U(IV) was more toxic than U(V). The survived

rats showed acute poisoning including nephrosclerosis, osteosarcoma, lung and kidney tumors, lung reticulolymphosarcoma and leukosis.

The behaviour of U^{233} oxide and U^{233} nitrate in rats was studied by Cooper et. al. (1982). The administered U translocated from lungs to blood at the same rate as U^{233} from $^{233}UO_2(NO_3)_2$ and $^{233}UO_2$. In blood plasma nearly 50% of the U^{233} was bound to transferrin, 25% to citrate and 25% to bicarbonate.

Rey(1983), studied the effects of Uranium compounds on the skin of male Wistar rats. In his report, he stated that the percutaneous absorption of UO_2, Uranyl nitrate, etc. was aided by a vehicle and known quantitites of various sized particle. U compounds were directly implanted in the subcutaneous (S.C.) tissues. Animals showed steady loss of body weight after high dose of UN. Histopathological damage to skin, hair follicles, adrenal gland were observed with higher concentrations of U in bone, teeth and kidney.

Pathological study on major damage from U compounds intoxication in rats, rabbits, dogs and mice was carried out by Sun(1983). UN inflicted damage to the kidney, especially to the second segment of the proximal convoluted tubule. The renal damage was more pronounced in rabbits followed by in rats, dogs and mice. Whereas, U induced liver damage was more pronounced in dogs. The hepatic damage was induced due to cell vacuolation, fatty acid degeneration, necrosis, hemorrhage etc.

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Lin-Shiau and Fu(1986), studied the antagonistic actions of UN on the presynaptic neurotoxins from snake venoms. According to them UO_2^{2+} antagonized the neuromuscular blocking action and phospholipase activity of neurotoxins.

Mode of inhibitory action of Zinc, Mercury, and Uranyl ion (2+) on 5'-nucleotidase of mouse hepatic microsomes was observed by Lin et al. (1986). The study shows that Mn^{2+} rather than Mg^{2+} is the metal ion activator for this enzymatic activity. Under physiological conditions, Zn^{2+} inhibited the enzymatic activity. Hg^{2+} exerted the inhibitory action by interacting with SH groups of the membrane proteins and competing with the metal ion activator for the binding site. Whereas UO_2^{2+} inhibited it by interacting with the membrane phospholipids.

Domingo et al (1987) observed the acute toxicity of U in rats and mice and studied the effect of route of administration.

Deposition and early disposition of inhaled Uranyl^{-233} nitrate and Uranyl^{-232} nitrate in the rat was studied by Ballou et al. (1986). The lungs of rats exposed to these two isotopes following inhalation of UN after 30 min contained, 7.23% of the total amount inhaled. Uranium was translocated rapidly from lungs and was retained mainly in skeleton, kidney and liver.

Absorption and biokinetics of Uranium in rats following an oral administration of UN solution was studied by Touch et al. (1987). Blood, kidney, liver and bone were analysed for U contents. Bone was found to be the primary tissue of it's position. Contents in skeleton and kidney tissues closely parallel each other from 15 min to 10 days after oral intake of known quantity. While in blood, the burdens reached a maximum within 30 min but declined rapidly afterwords.

B) **URANYL NITRATE TOXICITY AND KIDNEY** :

The effect of UN poisoning in rats on kidney plasma lactate dehydrogenase (LDH) and a-hydroxybutarate dehydrogenate (HBDH) isoenzymes were studied by Villie et al. (1972). According to them, the UN increased the plasma level of the isoenzymes, causing further increase in the HBDH isoenzymes than the LDH isoenzymes. While in kidney, the activity of LDH with the exception of LDH_4 and HBDH was found to be decreased.

Calcium metabolism in kidney mitochondria during acute U intoxication in mice induced an early and massive increase in the renal mitochondrial calcium (Carofoli et al., 1971).

In 1971, Rudniskaya and Mikhailo, studied the functional and morphological changes in the dog kidney, in later periods after UN treatment. The toxic symptoms showed breakdown of kidney function.

Treatment with Sodium bicarbonate or with either alone, alleviated the symptoms and increased their survival.

Renal and plasma chymotrypsin activity in the rat after experimental UN intoxication was determined by Gravelin et al.(1972). The activity in kidney showed a continuous decrease during the first 96 h, while plasma chymotrypsin increased during the first 24 h and leveled off thereafter.

Effect of varying doses of UN and saline loading on renal failure was observed by Ryan et al. (1973). The UN induced renal tubular necrosis. Saline loading designed to suppress the reninangiotensin system, ameliarated the azotemia, but not the tubular necrosis or tubular dysfunction. The protection afforded by saline loading suggested a role for renin-angiotensin system in the development of $UO_2(NO_3)_2$ induced acute renal failure (Ryan et al., 1973). Administration of UN led to brief period of Polyuria followed by progressive oligourea with death after 5 days (Blantz and Konnen, 1975).

In a similar study on dog by Kleinmon et al. (1975), it has been shown that the sodium excretion increased 5 fold and renal vascular resistance increased 3 fold along with a decrease in total renal blood flow and inulin clearance. Outer cortical ischemia occurred and plasma renin activity rose in the first hour and then slowly declined. Increased renin angiotensin system activity might

mediate the changes in renal hemodynamics and the consequent fall in glomerular filtration (Kleinman et al.,1975). Sodium ion variation in the distal tubule and renin activity in the juxtaglomerular apparatus in the UN induced acute renal failure (ARF) in the rat was studied by Flamenbaum et al. (1976). They have, further, evaluated the role of tubulo-glomerular feed back and noted the increase in renin angiotensin in plasma (Flamenbaum et al., 1977).

Renal glomerular and tubular lesions in rabbits treated with UN were observed by Gawlik et al.(1976). They observed non specific renal lesions in glomerular region, although the proximal convoluted tubule are considered to be the most susceptible part of the kidney.

Histopathological study on kidney of albino rat treated with UN was done by Goel et al(1980). The hisitological structure considerably altered after 19 days. Degeneration of epithelial cells and tubular wall was extensive. Severe renal lesions were observed after 27 days. During this period, the proximal convoluted tubule and glomeruli were most affected. The changes included vacuolization and loss of brush border.

The Glomerular filtration rate (GFR) decreased within 15 min of administration of UN but had no effect on the renal blood flow (Yamaguchi, 1980). There were no marked histological changes in the renal tissue, however, electron microscopy showed amorphous

glomerular deposites in the inner transparent basement membrane and endothelial cells (Yamaguchi, 1981).

The influence of UN upon tubular reabsorption and glomerular filtration in the perfused dog kidney was investigated by Nizet (1982). There was no significant change in urine and the decrease in fluid reabsorption was counterbalanced quantitatively by a reduction in GFR, only a small diminution of renal blood flow was observed by him.

UN induced sequential changes in renal morphology included focal brushborder loss and increased vacuolization in cortical proximal tubule. Middle and distal partion of proximal tubule were completely necrotic. Cells of initial part of proximal tubule accumulated large vacuoles in their cytoplasm. The distal nephron segments exhibited considerable cellular swelling and vacuolization (Haley, 1982; Sun, 1983).

Different doses of UN, UO_2 or UF_4 administered in rats, rabbits, mice and dogs revealed the renal damage in all of them, however, it was more pronounced in rabbits, followed by rats, dogs and mice, in that order (Sun, 1983), whereas, U induced liver damage to the maximum extent in dogs. Hepatic damage included cell vacuolation, fatty acid changes, necrosis, hemorrhage etc. (Sun 1983). Decrease in GFR and renal tubular sodium reabsorption rate accompanied with a decrease in the content of soluble protein-SH group

was observed. Pretreatment with glomerular stimulating hormone (GSH), checked the decrease in the soluble protein-SH group content (Nippon, 1983). Administration of EDTA immediately after the UN administration, showed a protective effect against the acute renal failure (Nippon, 1983).

The observed reduction in glomerular ultrafiltration coefficient in UN induced ARF in rats, can be prevented by prior administration of angiotensin converting enzyme inhibitor and normalized by plasma volume expansion and combination of these two treatments (Blantz et al., 1985).

C) **HEAVY METAL TOXICITY AND PROTEINS** :

Heavy metals binding to proteins extracted from rats were studied by Tykva et al. (1987). Cu-metaloprotein complex contents in Brown Norway rats were higher than in Fisher rats.

The effect of lead on protein and lactate dehydrogenase activity in hepatic slices cultured in vitro was observed by Kucharz and Stawiarska (1986). There was a decrease in the protein content and an increase in the lactate dehydrogenase activity. The fetal liver tissue was found to be more susceptible than the adult tissue.

Inhibition of rabbit reticulocyte lysate protein synthesis of heavy metal involved the phosphorylation of the α - subunit of the

eukaryotic initiation fructose-2 (Hurst et al., 1987). Heavy metal ion inhibitor protein synthesis in hemin supplemented lysate with biphasic kinetics. Shut off of protein synthesis occurred in conjugation with the phosphorylation of the α - subunit of the Eukaryotic initiation factor (βIF).

The U content in blood of some vertebrates was studied by Das et al. (1986). The experiments included animals like lata fish, toad and man. The lowest U concentration was found in toad. Segovia et al. (1986) carried out studies of U in the blood of two population samples which were similar in age and living patterns. Leukemia patients showed a higher concentration of U in blood. No change occurred in radiation exposed workers.

Changes in blood serum protein spectrum in white rats from inhalation and exposure to certain ferrites, were observed by Aleshina and Grin (1986). In rats exposed to Ba ferrite, Zn ferrite, Ni-Zn-ferrite, changes in relative composition of proteins of blood serum were common and the same were used for the detection of toxic levels of industrial air pollutants.

Effect of dietary fibres of vegetables on the content of sulfhydrial groups in rat blood and organ under cobalt poisoning was observed by Livshits (1987). Cobalt used as Cobalt Chloride $COCl_2$ decreased the SH concentration in blood serum, kidney liver, spleen and muscle for two months, after which a recovery was observed.

Changes in plasma proteins in rat treated for short periods with hepatotoxins or with agents which induce cytochrome P-450 isoenzymes were observed by Makarananda et al. (1987). They used centrilobular hepatotoxin. There was marked increase in the minor α_1-glycoprotein in chemically induced mitosis but not in mitosis following liver damage. Two major α_1-glycoproteins were reduced in all forms of liver damage. There were indications of specific protein response to allyl alcohol, to inducers of microsomal mixed function oxidases and to inducers of peroxisome proliferation.

Effect of Cadmium on synthesis of acute phase proteins was observed by Zak(1987). According to him the formation rates of fibrinogen and seromucoids of blood plasma were several fold above controls. Cycloheximide or actinomycin D administered 4 h. before the Cd injection decreased the formation rates below controls but when administered 4 h. after the Cd administration, was unable to prevent stimulation of the protein formation by Cd. Thus, it can be said that Cd activates the transcription stage of the formation of acutephase proteins.

The effect of Cadmium on the activity and concentration of some bio-chemical components of the blood plasma in rabbits was studied by Rafay et al.(1987). There was an almost linear increase in the Cd content in kidneys and liver above controls. But blood serum concentrations of proteins and glycerides were low.

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High fibre content in food showed protective effects against co-poisoning.

Identification and quantification of protein-blood ligand in Uramic serum was done by Takeda et al. (1987). A major protein bound ligand isolated from serum of chronic hemodialysis patients was identified as 3-carboxy-4-methyl-5-propyl-2-furan-propionic acid. The concentration of the Furan-propionic acid in serum as observed with HPLC is markedly greater in chronic hemodialysis patients.

Kanwar and Sharma (1986) analyssed the fluctuations in serum proteins and enzymes in mouse following oral lead administration. The activity of serum ATPase fell significantly following Pb treatment in both the adults and neonates compared with significant elevation in the serum proteins and also in the activities of alkaline and acid phosphatase.

Mitane et al. (1987), carried out the experiment on Cadmium induced inhibition of protein secretion from liver and the effect and specificity among Cadmium, Copper and Zinc. They observed that the serum cholin-esterase (CHE) was lowered on first day after Cd challenge in non-pretreated and Zn pretreated rats. Serum glutamic-pyruvic and glutamic-oxaloacetic transminase were not to 260 µg/gm after 24 h. by pretreatment with Zn. Both Cu and Zn induced depression of serum CHE activity was accompanied by elevated glutamic-pyruvic transaminase activity(enzyme leakage); Cd did not

LIBR. BALASAHEB KHARDEKAR
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induce enzyme leakage (Mitane et al., 1987).

Effects of endotoxin on plasma albumin and fibrinogen synthesis rate in rabbits were measured by the carbonate method by Koj and McFarlane (1968). They observed that the injection of endotoxin markedly decreased the fractional rate of loss in the first few hours of the injected radioidine labelled fibrinogen and to a smaller extent of similarly labelled albumin from the plasma. The absolute rate of synthesis of albumin and fibrinogen increased in endotoxin treated rabbits.

III) **CHANGES IN SOME PROTEIN FRACTIONS OF PLASMA** :

Certain α_1-globulins of rat plasma are known to increase in concentration after tissue damage. This observation was made by Gorden and Louis(1969).

The effect of cortisol on the synthesis of rat plasma albumin, fibrinogen and transferrin was studied by Jeejeebhoy et al. (1972). A decrease in absolute synthesis of albumin, no change in that of fibrinogen and an increased fractional synthesis of transferrin were evidenced.

Weiss and Linder (1985) identified a new plasma protein involved in copper transport in female rats. According to them Cu follows a carefully prescribed path and upon entering the blood, binds to the new transport protein.

IV) **WORK ON UN TOXICITY FROM THIS LABORATORY :**

The laboratory of Animal Physiology, Department of Zoology, Shivaji University, Kolhapur has been engaged in an extensive work on Uranyl nitrate toxicity, Since, more than a decade. So far a good number of research articles have been published in International/National journals devoted to this area and presentation of work in International/National Conferences have been well appreciated. UN toxicity is the target areas of this laboratory, since, it is very potent in inducing ARF and so is alarmingly hazardous. As the rate of mortality by renal failure in the Uranium industiral workers was breathtaking, the scientist in the area of nephrotoxicity realized and acknowledged the necessity of the experimental ARF model anlogous to human ARF. So keeping pace with this, our laboratory has contributed to the understanding of pathophysiologic mechanism of ARF, it's etiology, diagnosis and the prognosis.

Sagare and Sawant (1980, 1981) have studied the alterations in lipid profile as a result of UN intoxication. They observed sudden elevation in the phospholipid and neutral lipid levels indicating the role of UN in lipid metabolism, and thus adipose tissue, the lipid storing depot was brought into focus. Gojer and Sawant (1985a, 1985b) described alteration in the lipolytic activity in adipose tissue of mice following a nephrotoxic insult by UN.

Gojer and Sawant(1986) reported the development of the tolerance in experimental animals to repeated injections of UN and it's relationship with cell repair. Hematological changes occurring due to induced UN toxicity were described by Patil et al.(1986). The pathophysiological changes accompanied with the progression of UN toxicity were studied in Kidney, liver and brain by Desai and Sawant (1986, 1988). ARF model has been established with sensitive diagnostic methods (Gojer and Sawant, 1986). In 1988 Gojer and Sawant studied the treatment of Dithiothreital (DTT) and synergistic effects of water diuresis, DTT and Dopamine. The efficiency of saline loading as an antidote in UN induced ARF is also discussed with reference to the hematologic profile by Gojer and Sawant (1989).

Effect of UN induced ARF and it's progression on erythropoietic tissue has been reported in detail by Kadam et al. (1990). The corpuscular derangement as a result of an on-going progression of ARF is most significant observation that our laboratory has reported (Gojer and Sawant, 1990; Garware et al., 1990). Such derangement also plays a crucial role in the anemia of chronic renal failure (Pawar et al., 1990).

Antidote effects on the Uranium toxicity were also taken into consideration in our laboratory. DTT in recovery of erythrocyte structural derangement has been reported by Kulkarni et al.(1990).

V) REASONS THAT LED TO PRESENT INVESTIGATION AND PLAN OF PROPOSED WORK :

The critical review of the extensive literature available on metal toxicity in general and that of UN in particular indicates that very little information is available on the histopathological alterations biochemical changes induced due to the metal toxicity and the mechanism of action.

Blood is the first and foremost medium that is exposed to the UN toxic effect. It is necessary to study the early effects of UN at the plasma level and on the formed elements. The observations on rat and mice under experimental intoxication with UN were found to induce severe anemia (Gojer and Sawant, 1985; Patil, 1986; Kulkarni, 1992 and Pawar, 1992) and corpuscular derangement (Gojer and Sawant, 1989; Kulkarni et al., 1990; Kulkarni, 1992; Gojer and Sawant, 1992); however, there is scanty information on the effect of UN on plasma composition (Clarkson,1956; Nakano and Sapporo, 1959: and Andrlikova and Wagner, 1969).

There is a controversy regarding the mechanism of action of UN on the plasma composition. It will be interesting to study the effects of UN on plasma proteins with a hope to obtain a better explanation of the mechanism of UN binding and transportation OF UN during it's intoxication. Heavy metals like Cd, Pb, Hg, Cu are known to form

ligands with protein in the process of transportation. Though it is known that Hg and Uranyl ions readily bind to serum albumins and stabilize the albumin; it is not known whether there is any effect on the other plasma components.

Taking into consideration all the above aspects, which indicate the significance of studies on UN induced alterations in plasma components; it was proposed to take up a through analytical line of research in UN induced changes in plasma proteins.

Renal and hepatic malfunctions are the major symptoms of Uranium intoxication. Thus, one can expect alteration in the plasma components in UN induced nephrotoxic animal. Similarly several metal ions interact with amino acids and proteins in biological system. It will be interesting to test the possible protein-UN interaction from the point of view of the surface reaction and configurational changes.

Keeping in mind all above aspects which indicated that plasma protein alterations occurred during UN induced acute renal failure, it was proposed to take up a detailed investigation on the effect of UN intoxication at blood level.

The aims of the proposed work thus were -

A) To induce acute renal failure in rat using suitable dose of UN.

B) To prepare a plasma sample for detailed analysis of plasma proteins.

C) To quantitatively estimate the total proteins from the plasma.

D) To separate protein components from the plasma by SDS-polyacrylamide gel electrophoresis.

E) To identify and detect the individual plasma protein components by studying protein profile of plasma.

F) To compare the protein profile of the treated animal with control and find out the alteration, if any, in the plasma protein components of the rat.