CHAPTER II

BACKGROUND INFORMATION

Renal functional abnormalities in clinical acute renal failure

Acute renal failure (ARF) is an abrupt deterioration of renal function characterized by retention of nitrogenous compound. The etiology of ARF is varied, but nephrotoxins and renal ischemia are major causes (Chew & DiBortala, 1982). According to text books on human pathophysiology, 80% of ARF is due the hemodynamics crisis ("shock"), whereas 20% can be classified under toxic or allergic renal insults (Buchborn & Nierenversager, 1979).

This review is largely concerned with the utility of the ischemic experimental models. Before discussing the experimental studies of ARF, a brief review of the findings renal functional abnormalities in clinical ARF is warrented. Characteristically, the patients have a sudden development of oliguria or anuria, a low urine-to-plasma (U/P) urea and creatinine ratio, a U/P osmolarity of unity, and a high fractional sodium excretion (Levinsky & Alexander, 1976 ; Levinsky, Alxander & Venkatachalam, 1981). It has been shown that patients with ARF developed a markedly reduced renal blood flow and a rise in renal vascular resistance (Stein, Lifschitz & Barns, 1978). ARF is not only characterized by oliguria or anuria, but also by isotonicity of urine. This failure to concentrate may continue even when oliguria end off and leads to a polyuric stage. During renal failure and for some time after, the kidney is not able to concentrate urine properly. However, the phase of recovery deserves emphasis since one of the primary characteristics of ARF is the reversibility of the lesion and the eventual return of all

renal functional parameters to essentially normal levels (Stein & Sorkin, 1976).

Ischemic Acute Renal Failure

Experimental models of ischemic-ARF which have been extensively studies e.g. renal artery clamping, glyceral injection, intrarenal norepinephrine infusion. A reduction in renal blood flow (RBF) have been uniformly found in the initial phase of these models.Arendshorst et al (1975) recently studied the effect of 1 hr of renal artery occlusion on renal hemodynamics in the rats. RBF was reduced by approximately 40% whereas RVR was still increased in thirty to ninety minutes after release of the occlusion. Daugharty *et al* (1974) also found a 40% decrease in superficial glomerular plasma flow 1 hr after partial renal artery clamping. Earlier work using the xenon washout method, found a 19 % decrease in RBF within 10 minutes after intramuscular glycerol administration, which progressed to a 73% fall at 24 hrs (Ayer, Granchamp, Wyler & Truniger, 1971). Similar finding have shown in other method such as hydrogen washout method and radioactive mircospheres (Chedru, Baethke & Oken,1972 ; Hsu *et al*, 1976). It has been shown that a dose of 0.25 ug/kg/min of norepinephrine infused into the renal artery causes a fall in RBF and urine flow to almost zero and remain at this level along with the length of infusion. After discontinuation of the drug, there is a slow restoration of blood flow which rarely exceeds 50% of the control value at 3 hrs (Mauk *et al*, 1977). If the infusion is given for 2 hrs in the dog, renal function is irreversibly damaged (Cox *et al*, 1974).

The degree to which the decreased RBF can contribute to ARF and also depends on the particular model that is studied. A general

consensus exists that RBF the decrement is not related to decreased GFR in the maintenance phase of ARF, regardless of model of ARF employed. This mechanism may involve an increase in preglomerular resistance, either alone or in association with a decrease in post glomerular resistance (Hsu & Kurtz, 1981). The precise pathophysilogic basis for the increase in RVR in various forms of ARF is still unclear. It has been proposed that endothelial cell swelling is a self-perpetuating process induce by renal ischemia and maintain by the loss of the ability to regulate all volume (Flores, DiBona, Bech & Leaf, 1972). This proposal was rejected by the subsequent experiments of Frega *et al*, (1976) in the rat, for the reason there was the rapid return of blood flow in the particular model of ARF under evaluation. However,utilizing histologic techniques revealed that more than 90% of straight proximal tubules were occluded by swollen blebs of desquamated proximal tubular microvilli (Donohoe, Venkatachalam, Bernard & Levensky, 1976). In addition to the obvious mechanical effects of tubular obstruction, several investigator suggested that tubular obstruction eventually led to afferent arteriole constriction with a consequential fall in intratubular pressure (Arendshorst, Finn & Gottschalk, 1974 ; Tanner & & Steinausen, 1976). This phenomenon would seemingly explain the eventual reduction in intratubular pressure in the renal artery clamped model and possibly other experimental form of ARF.

Reversible of Acute Renal Failure

Generally, recovery phase of ARF can spontaneously occur, but it spend a long time. Diuresis will cause during recovery phase of ARF. In brief review will consider to experimental of reversible of ARF. Ringer loading can also restore RBF in both the renal artery

clamping and norepinephrine models 24-48 hrs after the insult without a significant return of GFR (Cox *et al*, 1974). Similary, there was a marked increase in RBF after Ringer loading in the dog, but oliguria persisted in $HgCl_2$ treated dogs (Bachler *et al*, 1977). After 1 hr infusion of acetycholine into renal artery in norepinephrine-induced ARF of the rat caused recovery of RBF, but inulin clearance does not improve because of intratubular deposits that cause tubular obstruction (Conger ,Robinette & Guggenheim, 1981). Propanolol administration is found to result in a decrease in medulary hyaline cast of ischemic model (Solez *et al*, 1977) Flores *et al* (1972) found that renal ischemia, cell swelling, "no reflow" and subsequent renal dysfunction occuring after obstruction to the renal arteries were collected by the administration of hypertonic mannitol, hypertonic sodium sulfate. Renal dysfunctions, however, are unaffected by an equivalent expansion of the extra cellular fluid volume in either loaded with isotonic saline or isotonic mannitol (Flores *et al*, 1972) Bailey *et al* (1973) showed that pretreatment of rats with varying doses of furosemide protected against both the acute tubular necrosis (ATN) and ARF of all models except for glycerol where the ATN and ARF was aggravated. In various experiment models of ARF, administration of furosemide has been reported to prevent or to partially reverse the course of ARF (Patak, Fadem, Lifeschitz & Stein, 1976), to increase urine volume without changing renal function (Ufferman, Jaenike, Freeman & Pabico, 1975).

Reactive Oxygen Species

Reactive oxygen species (ROS) are formed by incomplete reduction of molecular oxygen. The full reduction of molecular oxygen within the cells leads to water formation and needs four electrons

($O_2 + 4H^+ + 4e^- \longrightarrow 2H_2O$) when the reduction is incomplete, the oxygen molecule can be converted into reactive by-products.

Reduction of molecular oxygen by one single electron produces the superoxide radical ($O_2 + 1e^- \longrightarrow O_2^{\cdot-}$), which is both a free radical, since it bears an unpaired electron in an outer orbital, and an anion, since it contains more electrons than protons. Superoxide radical ($O_2^{\cdot-}$) can act as either a good reducing agent (for example, it reduces ferricytochrome C) or a fair oxidizing agent and is capable of initiating chain reactions (McCord, 1985).

Then the superoxide anion gains a supplementary electron to from hydrogen peroxide ($O_2^{\cdot-} + 1e^- + 2H^+ \longrightarrow H_2O_2$). This reaction occurs spontaneously but is also catalyzed by an intracellular enzyme, superoxide dismutase (SOD). H_2O_2 that does not posses the chemical structure of a radical can be reduced by O_2 to form the highly reactive hydroxyl radical ($H_2O_2 + O_2 \longrightarrow {}^1O_2 + OH^- + OH\cdot$). This reaction, known as the Haber-Weiss reaction, occurs in the presence of a trace metal, usually Fe^{3+} acting as an oxidation-reduction catalyst. It has been proposed that the oxygen formed in the Haber-Weiss reaction may be the singlet oxygen ($^1O_2$) (Laurent & Ardaillou, 1986). This species represents an oxygen molecule in the excited state (e.g.,characterized by the shift of one of the two unpaired electrons on an orbital of higher energy with an inversion of spin). The resulting energy excess is then dissipated by thermal decay, light emission, or chemical reaction H_2O_2 is also the source of formation of powerful oxidants. In the presence of myeloperoxidase, an enzyme essentially present in the azurophil granules of the polymorphonuclear leukocytes (PMNL), and of a halide such as chloride, there is formation of hypochlorous acid. The contribution of iodide is uncertain, due to its low concentration in

the serum. Hypochloride is toxic in itself and can also interact with H_2O_2 to form $^1O_2$ ($OCl^- + H_2O_2 \longrightarrow {}^1O_2 + Cl^- + H_2O$).

Cellular defenses against ROS

Cells tolerate the production of ROS only if their concentration is maintained at a low level. This is achieved by intracellular detoxifying agents that are enzymes or scavengers. Both are present in the kidney. Two types of SOD that contain either copper and zinc or manganese at their active site and are located in the cytoplasm and the mitochondria, respectively (Chance, Sies & Boveris, 1979), catalyze the dismutation of $O_2^{\cdot-}$ into H_2O_2. In contrast with what is observed in the lung, renal SOD activity is not modified during hyperoxia (Crapo & Tierney, 1974). SOD is not really a detoxifying enzyme, since the product of its activity, H_2O_2, is a toxic agent. However, dismutation of O_2 is the first step of the enzymatic cascade leading to the complete inactivation of the ROS formed. The second step, which depends on catalase, corresponds to the transformation of H_2O_2 into water ($2H_2O_2 \longrightarrow 2H_2O + O_2$). Catalase has been found in the cytosol and subcellular organeles such as the peroxisomes of the kidney (Chance, Sies & Boveris, 1979). Its activity is inhibited in the presence of high concentrations of NaCl (Miyahara & Samejima, 1981). Glutathione peroxidase (GSHPX) activity also results in the protection of the all from an excess of H_2O_2. Several minerals, such as selenium, zinc, and copper, or vitamins, such as riboflavin and tocopherol (vitamin E), are essential in the defense of the cells against the oxidative damage because they act as catalysts of the detoxifying enzymes or as endogenous scavengers. Chronic deficiency of vitamin E or selenium is associated with an increase in the rate of lipid peroxidation in the kidney (Freeman & Crapo, 1982).

Role of ROS in Ischemic acute renal failure

A number of studies indicate that ROS are produced in ischemic tissues and therby contribute to cell damage. It was first thought that ischemia and hypoxia modified the mitochondrial respiration so that the rate of O_2^- formation was increased due to the diminution of the fraction of oxygen that was completely reduced to water. The major source of superoxide in postischemic tissues appears to be the enzyme xanthine oxidase. This enzyme was the first documented biologic source of the superoxide radical (McCord, 1985). It is widely distributed among tissues ; the intestine, lung and liver are particularly rich sources in most species. The enzyme is synthesized as xanthine dehydrogenase (type D). This form appears to account for about 90 percent of the total activity in a healthy tissue (Roy & McCord, 1983). The dehydrogenase cannot transfer electrons to molecular oxygen to form hydrogen peroxide or superoxide, but can reduce NAD^+ (nicotinamide-adenine dinucleotide), as follows :

$$\text{Xanthine} + H_2O + NAD^+ \xrightarrow{\text{type D}} \text{uric acid} + NADH + H^+$$

When most tissues are homogenized without special precautions, the dehydrogenase converts rapidly to the oxidase (type O) as a result of sulfhydryl oxidation or limited proteolysis (Della Corte & Stirpe, 1972). rapid freezing of tissues in liquid nitrogen, followed by homogenization in ice cold buffers containing agents that inhibit proteases and sulfhydryl oxidation, can prevent this conversion. The oxidase can use molecular oxygen instead of NAD^+, producing superoxide or hydrogen peroxide or both, as follows :

$$\text{Xanthine} + H_2O + 2O_2 \xrightarrow{\text{type O}} \text{uric acid} + 2O_2^- + 2H^+$$

Although it had been known for some time that this conversion was possible, early investigations produced no data suggesting that the process could occur in vivo or have pathophysiologic importance. We have found that the conversion of xanthine dehydrogenase to xanthine oxidase does, infact, occur in vivo in ischemic tissues (Roy & McCord,1983).

The process of the conversion of type D activity to type O activity in vivo begins when the decrease in blood flow to a tissue is sufficient to limit oxygen availability for requisite production of ATP. As the cell's energy charge drops, it is no longer able to maintain proper ion gradients across its numbers, and this precipitates a redistribution of calcium ions. The elevated cytosolic calcium concentration, we believe, activates a protease capable of converting the dehydrogenase to the oxidase. Concomitantly, the depletion of the cell's ATP results in an elevated concentration of AMP. The AMP is catabolized to adenosine, inosine, and then hypoxanthine. The buildup of hypoxanthine have been observed in the kidney of rats with ischemic ARF (Paller, Hoidal & Ferris, 1984). Hypoxanthine, as well as xanthine, serves as an oxidizable purine substrate for xanthine dehydrogenase or oxidase. Hence, during ischemia, two important changes occur in tissue : a new enzyme activity appears, along with one of its two required substrates. The remaining substrate required for type O activity-molecular oxygen is supplied during the reperfusion of the tissue ; with it comes a burst of superoxide radical and hydrogen peroxide production. This sequence of events is diagrammed in Fig. A

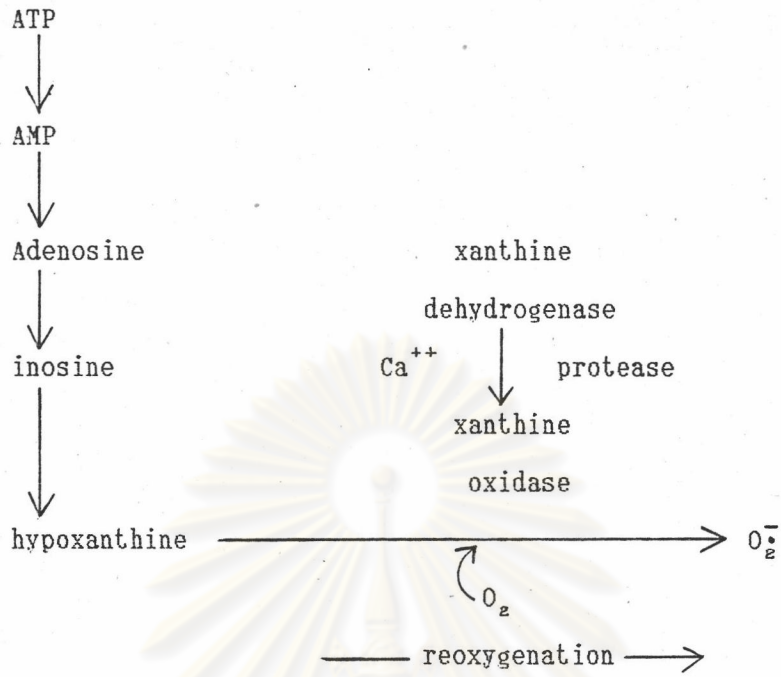

Fig. A Proposed Mechanism for Ischemia-Induced Production of Superoxide

In Vitro Effects of ROS on the Kidney

To date, the effects of ROS have been essentially studied only in the glomeruli. ROS alter the glomerular components, particularly the glomerular basement membrane (GBM), and also modify the glomerular synthetic functions.

Degradation of GBM and renal cytotoxicity of ROS. During nephrotoxic glomerulonephritis, PMNL insert themselevs between the GBM and the overlying endothelial cells. This suggests that GBM components can stimulate PMNL and that the various toxic substances released by the activated PMNL, particularly the ROS, can diffuse into the GBM and degrade its constituents. There was in parallel a release of lysosomal enzymes from PMNL, one of these enzymes degrading the collagen moiety of the GBM.

Renal tubular cells are also altered in the presence of an excess of ROS. Exposure to H_2O_2 of freshly isolated rat cortical cells produced the death of > 50% of these cells as estimated by trypan blue exclusion. Ionophore A 23187 and E.coli endotoxin were also, and their effects were markedly inhibited by SOD and catalase (Keane, Van Asbeck, Gekker & Peterson, 1985). H_2O_2 added directly or generated by glucose oxidase increased the transepithelial electrical conductance of cultured MDCK cells, which are derived from the dog renal cortex. This was interpreted as the result of an increase of the permeability of the paracellular pathway. There were also alterations in organization of the cell cytoskeleton, particularly in the area of the cell-to-cell junctions (White, Crawford, Patt & Lad, 1976).

Toxic effects of ROS

It is very likely that ROS generated under these conditions contribute to the cell injury. ROS released from activated neutrophils and macrophages or from the renal cells themselves may induce several types of cell damage. The cell membranes contain high amounts of polysaturated fatty acids, which react with ROS to form peroxide derivatives (R-O-O-H). These products are chemotactic lipids that may cause further accumulation of inflammatory cells of degradation products are formed that are considered to be quantitative indicators of the lipid peroxidation. (Kellogg & Fridovich, 1975). These include, in addition to lipid hydroperoxides and hydroxy fatty acids, ethane, penthane, and malondial dehyde (MDA). For example, lipid peroxidation associated with vitamin E or selenium deficiency has been estimated using MDA formation (Doni *et al*,1984), and that following renal ischemia has been estimate using ethane production. (Paller & Hebbel, 1986). Lipid peroxidation results in alteration

of membrane paroperties such as fluidity, ion transport, and enzyme activities. It has been reported that endogenously generated ROS produced an increase in passive K^+ permeability in red cells. Such a change in cell permeability could have a prominent effect on ion transport in the kidney (Maridonneau, Braquet & Garay, 1983). In addition to oxidation of the membrane lipid, other specific cellular vents result from ROS injury, particularly decrease in ATP levels (Sragg *et al*, 1985) and inactivation of intracellular enzymes by attack of their-SH or $-NH_2$ groups. These effects should alter the transport capabilities of the renal cells. The hypochlorous acid formed by interaction between H_2O_2, Cl^-, and myeloperoxidase produces halogenation of proteins, which is another mechanism of tissue injury (Couser *et al*, 1986). Cell lysis may occur in the presence of high concentrations of ROS (Fridovich, 1978).

Tetrachlordecaoxide (TCDO)

Tetrachlordecaoxide is an aqueous solution containing a non-metallic biocatalytically activated oxygen carrier in the form of a complex, stable only in solution (Fig.B).

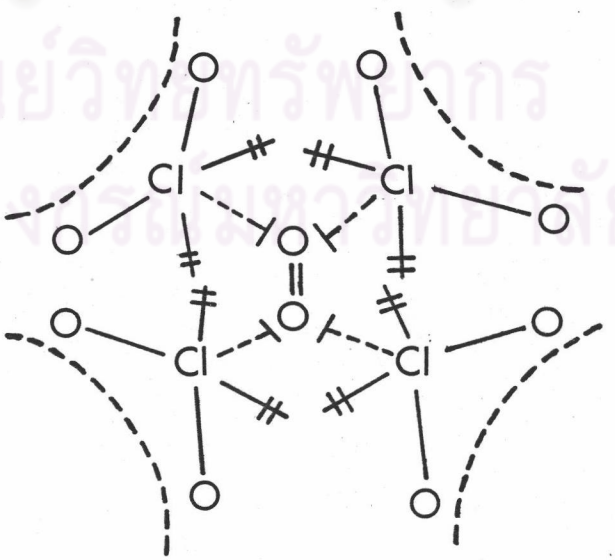

Fig. B Structural Formular Tetrachlordecaoxide

It has no chemico-pharmacological precursors. When acted upon by biocatalysts, the oxygen complex is converted completely into the physiological metabolites, oxygen and chloride. In invivo studies, quantitative oxygen and chloride ions and released by the influence of catalytic enzyme. There by no toxic oxygen combinations such as superoxide or peroxide radicals develop. In in vitro studies under the catalysis of certain hemeproteins such as peroxidase (s), myoglobin Fe^{2+} or Fe^{3+} hemoglobin (but not with catalase), TCDO lead to the formation of an active oxygen species. This agent does not posses the same biological and chemical properties as the aggressive free OH^{-} radical, the superoxide anion, hydrogen peroxides and singlet oxygen. The initiation of increased oxygen supply in hypoxic tissue by TCDO is a complex process which may occur as in Fig.C.

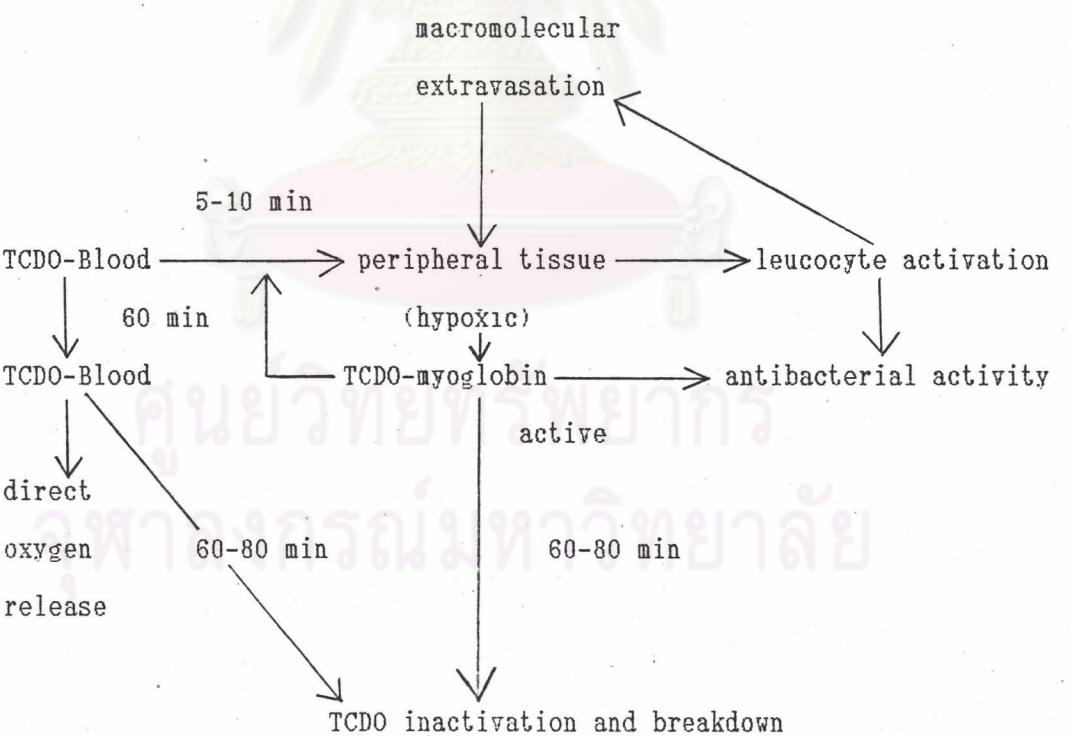

Fig. C The scheme of hemcatalyzed activity of TCDO (The times indicated refer to reactions in the in vitro system)

Myoglobin reacts with TCDO without an activation phase, whereas blood requires a period of preincubation of about 45 min in order to achieve comparable rates. In both cases, the product of the reaction is a biologically relevant oxidant which can be compared with the mycloperoxidases-catalysed reaction and, however, does not lead to the production of singlet oxygen. TCDO also forms an active oxygen species which is very similar to the product of the polymorphonuclear leucocyte (PMNL) myeloperoxidases, so the efficiency of phagocytosis of granulocytes in whole blood is increased. In addition to the bactericidal (bacteriostatic) properties in inflamed tissue, TCDO also leads to increased blood supply, which likewise appears to occur via the agency of active oxygen species (Youngman, Wagner, Kuhne & Elstnerm 1985.)