CHAPTER II

BACKGROUND INFORMATION

Vanadium

Vanadium is a trace element in a number of the Vb group that occurs naturally in the earth's crust. An atomic weight is 50.94. It was discovered by the Swedish scientist N.G., Sefstrom in 1830 and named after the Scandinavian goddess "Freya Vanadis." (Grantham, 1980; Jandhyala and Hom, 1983). It is wildly distributed in the environment and occurs in varying concentration in soil, water, air, plants and animal tissues. It is probably an essential trace element for animals. Vanadium is utilized as an alloy in the steel industry, as a catalyst in the chemical industry and in the production of electronics and ceramics. Exposure to vanadium is of concern because it increased concentration near industrial operations, its occurence as a by-product of petroleum and metallurgical refinement, and its subsequent accumulation in the environment (Phillips, Nechay, and Heidelbaugh, 1983).

Chemistry of vanadium compounds.

Vanadate is a salt of vanadium compounds. Potential oxidation states for vanadium range from -1 to +5, however two valence states are relevant, the tetravalent, VO^{2+} (+4) and the pentavalent, VO_3^- (+5). (Nechay, 1984; Nechay et al. 1986). In plasma, vanadium apparently exists as vanadate (metavanadate), VO_3^- (+5 oxidation state) and inside cells in reduced form as vanadyl VO^{2+} (+4 oxidation state) (Nechay, 1984). Vanadyl is a common intracellular form in animal tissue, has been shown to bind to proteins, and is generally thought to be ineffective as an inhibitor of the Na^+ pump. (Phillips et al, 1983). Cantley et al.(1977) have reported that vanadate is one of the most potent known inhibitors of Na^+-K^+ATPase, and suggested that it may be a physiologic regulator of the Na^+ pump.

Effects of vanadate on enzyme systems

Cantley et al (1977, 1978) demonstrated that vanadate is a potent inhibitor of the Na^+-K^+ATPase. In the present time, it is found that the action of vanadate is not a selective inhibitor of Na^+-K^+ATPase, but it also blocks many other enzymes system (Nechay, 1984; Nechay et al., 1986). It was shown that Ca^{++}-ATPase from human red cell membranes was inhibited with high affinity by vanadate (Jandhyala and Hom,

1983, quoting Bond and Hudgins; Baraabin, Garrahan, and Fega). Similar inhibition of Ca^{++}-ATPase by vanadate has been demonstrated in various tissues : squid axons and rat brain synatosome; kidneys; the sarcoplasmic reticulum of the hearts of pigs; dogs and rabbit skeletal muscle. (Jandhyala and Gary, 1983, quoting Rossi; Garrahan and Fega; Baker and Singh; Wang et al; Shigekawa and Pearl). Furthermore, vanadate can inhibit H^+-K^+ATPase in the microsomal gastric mucosa (Faller et al, 1982) and H^+, K^+ATPase of Streptococus faecalis (Furst and Solioz, 1985). Moreover, Arruda et al. (1981) have shown that the H^+ pump in the turtle urinary bladder was inhibited by vanadate. Recently, it has been shown that vanadate also inhibits H^+-K^+ATPase in distal nephron segments (Doucet and Marsy, 1987; Gary and Narang, 1988) and H^+-ATPase in turtle bladder (Youmans and Barry, 1989). In addition to, it can inhibit other enzymes such as an alkaline phosphatase, phosphotyroryl protein phosphatase, the contractile protein ATPase, dynein and ect. (Nechay et al, 1986). In 1986, Nechay suggested that vanadate in living animals causes diverse effects in different organ systems, probably because vanadate inhibits a variety of phosphatase enzymes including Na^+-K^+ATPase, Ca^{++}-ATPase, H^+-K^+ATPase, and H^+ATPase; and in the vanadyl form stimulates adenylate cyclase.

Distribution of vanadium

Vanadium is distributed in tissue and body fluid. In the "reference human" the total-body pool of vanadium is about 100 µg with a dialy intake of 10-60 µg. Approximate wet tissue concentration (in nanograms of vanadium per gram) are; liver, 13; kidney, 5; bone, spleen, and thyroid, 3; brain, fat, milk, colostrum, bile, and urine, less than 1; lungs and hair, 12 - 140 (with upper range values in city dwellers)(Nechay, 1984; Nechay et al, 1986).

The average of mean human serum concentration of vanadium is about 0.35 ng/ml or about 7×10^{-9}M (Nechay, 1986). It is excreted mainly in the feces and urine, with mean urinary output per 24 hours is 10 µg (Nechay, 1984). Nechay (1984) reviewed the following values in other mammals and showed that bone, 20-40 (pig, sheep); bone marrow, less than 1 (pig); liver, 2-10 (beef, pig, rat); kidney, 9-34 (rat, pig, dog, rabbit); heart, less than 1-9 (pig, rat, rabbit); skeletal muscle, less than 1-14 (beef, pig, rabbit, horse); brain, (1-3 (rat, cow); lung, 5-25 (rabbit, beef); fat, less than 1-2 (pork); butter, 1; milk less than 1-3; plasma, 2-5 (rat); gelatin, 9-43; whole mouse, 66. As previously indicated, vanadium is found in animal tissues in trace amounts; however, the highest concentrations are commonly detected in renal tissue (Phillips et al,

1983, quoting Oberg et al; Parker et al.), primarily in the cortex (Balfour, Grantham, and Glynn, 1978a; Grantham, 1980; Kumar and Corder, 1980). Although vanadium is belived to be an essential trace element for higher animals, its biological and toxic mechanism of action are not understood (Phillips et al, 1983). The toxicity of vanadium has been reported by many researchers (Jandhyala and Hom, 1983; Philips et al, 1983; Nechay, et al, 1986). It is largely associated with acute or chronic respiratory exposure. Green tongue, rhinitis, pharygitis, and bronchitis are common. Clinical symptoms of gastrointestinal distress, fatigue, pneumonitis, bronchopneumonia, cardiac palpitation, and kidney damage have also been linked with industrial exposure (Phillips et al, 1983, quoting Waters; Hammond and Beliles). Vanadium has been shown to produce other physiological effects such as disturbance of the central nervous system and cardiovascular changes, as well as metabolic alteration in experimental animals (Phillips et al, 1983, quoting Waters), the mechanism of which have not been adequately defined. Moreover, because vanadium accumulates in renal tissue to a large extent and is principally eliminated in the urine. Thus, the kidneys may represent a major site of toxic and pharmacological action of vanadium.

Effects of vanadate on renal functions

Balfour, Grantham and Glynn (1978a, 1978b) have demonstrated that vanadate induced profound diuresis and natriuresis when administered into the rats. Studies of Roman et al. (1981) have shown that glomerular filtration rate and arterial pressure were not altered and the observed effects were mainly due to reduction in tubular reabsorption of water and electrolyte. Day et al. (1980) also demonstrated dose dependent increased in diuresis and natriuresis after vanadate administration in conscious rats and these effects are not due to change in either blood pressure or glomerular filtration rate. These studies also showed that vanadate did not alter urinary potassium excretion. Kumar and Corder (1980) have reported the effects of vanadate in isolated perfused rat kidney. In contrast to *in vivo* studies, varying concentrations of vanadate in the perfusate (0 to 32 μM) produced a dose dependent rise in glomerular filtration rate, urine flow rate, renal vascular resistance and an inhibition of sodium reabsorption. Since vanadate produced simultaneous rises in glomerular filtration rate and vascular resistance, a post capillary vasoconstrictor effect of vanadate has been postulated by these investigators. Earlier studies *in vitro* as well as *in vivo* and micropuncture experiments suggested that the diuretic and natriuretic effects of

vanadate were due to its ability to inhibit Na^+-K^+ATPase in the proximal tubule. Grantham and Glynn 1979; Day et al., 1980; Higashi and Bello-Reuss, 1980). However, evidence was available to suggest that vanadate inhibited tubular reabsorption in virtually all segments of the nephron (Westenfelder, Hamburger and Garcia, 1981). Vanadate also enhanced excretion of calcium, phosphorus, sulfur, magnesium and chloride in rats (Roman et al. 1981). Day et al. (1980) reported that vanadate decreased urine potassium concentration but increased potassium excretion. Kumar and Corder (1981) found that vanadate had no significant effect on either potassium concentration or excretion. The result of Roman et al. (1978) indicated that potassium secretion by distal segments of the nephron was not directly affected by vanadate but would vary with change in sodium concentration in the tubular fluid.

The preceeding discussion was based on the data obtained from renal function studies conducted in rat kidneys, *in vitro* as well as *in vivo*. In contrast to these data, investigators failed to demonstrate similar diuretic and natriuretic effects of comparable doses of vanadate in anesthetized cats and dogs. Vanadate infusions in anesthetized cats and dogs produced dose dependent increased in renal vascular resistance accompanied by a fall in glomerular

filtration rate, renal blood flow, urine output and electrolyte excretion (Larsen, Thomsen, and Hansen, 1979; Lopez-Novoa, Mayol, and Martinez-Maldonado, 1982). These studies in cats and dogs suggested that, renal vascular effects of vanadate is predominate due to tubular effects; hence, changes in systemic and renal hemodynamic would determine net changes in urine output and electrolyte excretion.

H^+ translocating ATPase

Several lines of evidence indicated that distal nephron acidification occures by means of primary electrogenic proton translocating ATPase that is sodium independence (Koeppen, Giebisch, and Malnic, 1985). The predicted sodium independency of this acidification event has been demonstrated in studies conducted in turtle urinary bladder (AL-Awgati, 1976), rabbit cortical collecting tubule (Koeppen and Helman, 1982) and medullary collecting duct (Stone et al, 1983). In all instance, distal urinary acidification is found to produce by means of a primary electrogenic event which is directly modulated by aldosterone. H^+ translocating ATPase are used by cells either to generate large pH gradients (e.g., > 6 pH unit across the gastric epithelium) or to transduce the energy in pH gradients to ATP (Ives and Rector, 1984). There are three types of H^+-translocating

ATPase that has been divided by Ives and Rector (1984), Stone and Xie (1988).

1. The first is called F_oF_1ATPase, named for the coupling factors F_0 and F_1 found in early research on oxidative phosphorylation (Sachs et al, 1982). It is found in bacterial cells, mitochondria and chloroplasts. This type of H^+ATPase typically is inhibited by less than 1 μM NN'-dicyclohexylcarbodiimide (DCCD). The F_oF_1 H^+ATPase in mitochondria in a few bacterial are also inhibited by oligomycin.

2. The second type is called E_1 - E_2 ATPase (Stone and Xie, 1988) that has been found in gastric mucosa, H^+-K^+ ATPase (Sachs et al, 1982); Neurospora plasma membrame proton translocating ATPase (Ives and Rector, 1984, quoting Bowman). ;H^+-K^+ ATPase in distal nephron segments that similar to H^+ - K^+ ATPase in gastric mucosa (Doucet and Marsy, 1987; Gary and Narang, 1988) and turtle urinary bladder (Youmans and Barry, 1989).

Like the Na^+ - K^+ ATPase and Ca^{++} ATPase, these enzymes have as part of their catalytic cycle an acyl phosphate intermediate (Stone and Xie, 1988). Vanadate and omeprazole serve as the inhibitor of these enzymes by substituting for phosphate during the catalytic cycle (Doucet and Marsy, 1987; Gary and Narang, 1988; Stone and Xie, 1988).

3. The third type of H^+ translocating ATPase is NEM sensitive H^+-ATPase which has resistance to both the F_0F_1 type inhibitor, oligomycin and the E_1-E_2 type inhibitor, vanadate. In contradistinction, this pump is inhibited by the water soluble sulfhydryl reactive reagent, N-ethylmaleimide (NEM) (Stone et al, 1983; Rudnick, 1986; Mellman, Fuchs, and Helenius, 1986). It was found in the turtle bladder (Dixon and Al-Awqati, 1979), bovine, rat and human kidney medulla (Gluck and Al-Awqati, 1984; Kaunitz, Gunther, and Sachs, 1985; Diaz-Diaz et al, 1986).

Effects of vanadate on H^+ translocating ATPase and urinary acidification

Steinmetz, et al. (1980) have demonstrated that the acidification pump of the luminal cell membrane of the turtle bladder had the H^+-ATPase characteristics that differ from mitochondrial ATPase in that H^+ transport was oligomycin-insensitive and vanadate-sensitive. It also showed hat vanadate inhibited H^+ transport by a mechanism of uncoupling. Arruda et al. (1981) have reported that vanadate had a number of effects that were capable of inhibiting acidification by the turtle bladder. Among these effects, the inhibition of urinary acidification could be the result of inhibition of the H^+ pump, interference with utilization of ATP by the pump, or

other metabolic effects. Sallmam et al, (1986) have also studied about plasma membrane proton ATPase from human kidney by isolated a plasma membrane fraction from human kidney cortex and medulla which contained H^+-ATPase activity. The plasma membrane fraction showed ATPase activity which was sensitive to dicyclohexyl carbodiimide (DCCD) and N-ethylmaleimide (NEM). Moreover, this ATPase acitivity was also inhibited by vanadate, 4,4′-diisothiocyano-2,2′-disulfonic stilben and $ZnSO_4$. Acidification was not altered by replacement of Na, or K, but was critically dependent on the presence of chloride. They summarized that the plasma membrane fraction of the human kidney cortex and medulla contained a H^+ ATPase, which was similar to the H^+-ATPase described in other species, and postulated that this H^+-ATPase may be involved in urinary acidification. Recently, Youmans and Brodsky (1987) have demonstrated that the active transport of H^+ in vesicle had two components, one of which was inhibited by low concentrations of vanadate (apparent I_{50}, 45 nanomolar) and one which was unaffected by concentration as high as 1×10^{-4}M. In additional,they further confirmed the existence of two district ATP-dependent proton transport process in isolated epithelial cell membrane from the turtle bladder (Youmans and Bary, 1989). It seems likely that these processes correspond to the vanadate-insensitive and vanadate-inhibitable portions of the

transepithelial acidification current found across the intact bladder. Their works indicate that one process is inhibited by low concentrations of NBD-Cl but is insensitive to vanadate, and their could be due to a vacuolar-type H^+-ATPase. This class of enzyme has been described in number of intracellular organelles. Proton pump-containing cytoplasmic membrane vesicle, which are thought to be exocytotically inserted into the apical membrane upon stimulation of acidification in the turtle bladder and mammalian collecting duct epithelia. The other process, less sensitive to NBD-Cl but inhibited by low vanadate concentrations, appears analogous to the E_1-E_2 type constitutive plasma membrane H^+ transporters found in other tissues. They suggested that the availability of inhibitors selective for each process in isolated membrane will aid in determining the role of each of these transport processes in acid secretion by the intact bladder.

Effects of vanadate on H^+-K^+ATPase and urinary acidification.

Doucet and Marsy (1987) have demonstrated that K^+-ATPase which belongs to the E_1-E_2 class of ATPase similar to H^+-K^+ ATPase described in gastric mucosa and colon was inhibited by vanadate and omeprazole, and was insensitive to ouabain. Moreover, K^+ ATPase activity was detected highest in the connecting tubule, intermediate in the cortical collecting tubule, lowest in the outer medullary collecting tubule, and no detectable in all other nephron segment. They suggested that the possibility that K^+ reabsorption and H^+ secretion might be linked functionally which supported by the fact that acidosis is accompanied with alteration of luminal membrane of intercalated cells (Hagege, Gabe, and Richet, 1974), similar to these observed during K-depletion (Doucet and Marsy, 1987, quoting Sach et al.) Gary and Narang (1988) have also reported that K^+-ATPase present in the connecting tubule, cortical collecting duct and medullary collecting duct had some properties in common with gastric H^+-K^+ ATPase and was inhibited by vanadate,omeprazole and SCH 28080 (relatively specific inhibitors of gastric H^+-K^+ ATPase). Furthermore, the recent study of Wingo (1989) was demonstrated that omeprazole fully inhibited both proton secretion and K absorption in the outer medullary collecting duct of

rabbits conditioned to a low-K diet. These observations were consistent with the hypothesis that omeprazole inhibited a primary active pump that energized the active exchange of protons for K ion across the apical membrane. This model is similar to that developed to explain proton secretion by the gastric mucosa in which omeprazole inhibits active H^+ secretion and active K^+ absorption (Wingo, 1989, quoting Reenstra et al). Indeed, the collecting duct of both the rabbit and the rat has K-ATPase activity that exhibited inhibition characteristics similar to the gastric proton pump (i.e., insensitive to ouabain but inhibited by vanadate and omeprazole) (Wingo, 1989, quoting Imbert-Teboul; Doucet and Marsy, 1987; Gary and Narang, 1988). This may suggest that omeprazole also inhibits and electrogenic proton ATPase (H-ATPase), which has been proposed to be the major enzyme-responsible for luminal acidification in this segment.

It can be summarized that vanadate may inhibit K^+-ATPase in distal nephron segments which similar to H^+-K^+ ATPase in gastric mucosa and H^+ ATPase in turtle urinary bladder, all are E_1-E_2 type of H^+ translocating ATPase. The physiological role of these enzymes are not known at the present time. It is possible that it may be involved in K^+ reabsorption and/or H^+ secretion in distal nephron segments that

plays a role of distal urinary acidification. Thus, further study and investigation on the effect of vanadate on these enzymes and urinary acidification need to be searched and discussed.