CHAPTER V

DISCUSSION

It has been shown that vanadate infused into renal artery and intravenous continuously produced an increased in mean arterial blood pressure significantly in normal rats, cats and dogs (Larsen et al., 1979; Day et al., 1980; Inciarte et al., 1980; Lopez-Novoa, 1982). Vanadate also caused to exhibit vasoconstriction in most mammalian vascular bed and raise blood pressure (Inciarte et al.,1980;Lopez-Novoa et al., 1982, quoting Jackson; Larsen and Thomson). In this study vanadate did not increase in mean arterial blood pressure in saline control group,it may explain that firstly, the bolus dose of vanadate used is small and can not reach the systemic vascular effects to induce vasoconstriction. Secondly, the extracellular pH is not enough to accentuate action of vanadate, because the solution chemistry for vanadate ionic species is complex and dependent on pH and vanadium concentration. The VO_4^{3-} (vanadate) ion exists in basic solution; however, as the pH is lowered, protonation ($H_2VO_4^-$) and dehydration can occur, forming di- and trivanadate ions such as the isopolymeric trimer $V_3O_9^{3-}$ (active form) at vanadate concentration of less than 10^{-3}M. (Phillips et al., 1983). Crans et al. (1989) suggested that it is

possible that pH may be a key factor be consider when examining the effects of vanadate in biological system, and one must be aware that subtle pH changes alter the strength of the vanadate-protein interaction.

In our results, there was gradual increase in mean arterial blood pressure obviously after injection of vanadate into the renal artery in metabolic acidosis group. The cellular mechanism of vanadate induced vasoconstriction is not clear. The possible mechanism are firstly, it may inhibit Na^{+}-K^{+}ATPase resulting of transmembrane gradient of Na^{+} concentration, which would slow the Ca^{++} efflux through the Na^{+}-Ca^{++} exchange mechanism in the membrane. The increase in intracellular concentration of calcium would lead to contraction of smooth muscle (Lopez-Novoa et al., 1982, quoting Barchrd). Secondly, vanadate also inhibits the sarcoplasmic Ca^{++}-ATPase which is considered to be responsible for active Ca^{++} uptake leading to a reduction of cytoplasmic calcium concentration (Lopez-Novoa et al., 1982, quoting O'Neal et al.; Wang et al.). This effect leads to increase calcium concentration in the cytosol that induces vasoconstriction. Thirdly, vanadate has been reported to stimulate norepinephrine release (Nechy, 1984, quoting Torok et al).

It has been demonstrated that intravenous and intrarenal arterial infusion of vanadate induced renal vasoconstriction in cats and dogs. (Larsen et al., 1979; Lopez-NoVoa et al., 1982; Benabe, Cruz-Soto and Martinez-Maldonado, 1984). It this present study, it was found that effective renal plasma flow and glomerular filtration rate decreased significantly in metabolic acidosis, whereas a slight reduction without statistically significance in saline control group. The increase in peripheral vascular resistance and renal vasoconstriction by vanadate causes a decrease in effective renal plasma flow and glomerular filtration rate (Larsen et al., 1979; Lopez-NoVoa et al., 1982; Benabe et al., 1984). However, the urine flow rate slightly diminished in metabolic acidosis but not indicate statistically significance. There was a little increase in urine flow in saline control, while the decline of effective renal plasma flow and glomerular filtration rate was seen. Thus, these results suggest that vanadate may have effect to decrease the renal sodium and water reabsorption.

The present data indicates that after vanadate injection, the fractional excretion of sodium is elevated in both groups. This result is similar to previous experiment both *in vivo* and *in vitro* in rats leading to an important increase in sodium and water excretion by the kidney (Balfour et al., 1978a, 1978b;

Higashi and Bello-Reuss, 1980; Day et al., 1980; Kumar and Corder, 1980; Westenfelder et al., 1981). The mechanism of vanadate produced natriuresis has not yet been established and could possibly result from many different factors, including effects on adenylate cyclase (Schwabe et al., 1979). It is presently more apparent from the avilable literature that the mechanism of natriuresis and diuresis may be involved by vanadate's potent inhibition of renal Na^+-K^+ATPase in proximal tubule (Balfour et al., 1978a, 1978b; Higashi et al., 1980; Day et al., 1980; Westenfelder, 1981). Moreover, in metabolic acidosis group, the increase in fractional excretion of sodium is more than in saline control. This results may be caused by acute metabolic acidosis, that could also reduce proximal NaCl reabsorption a modest natriuresis. (Narins et al., 1985, quoting Cogan and Rector).

The effect of intrarenal arterial injection of vanadate produced an increase in fractional excretion of potassium in both groups. This phenomenon has also been shown in the previous studies in renal rats (Day et al., 1980; Roman et al., 1981). The report of Roman et al. (1981) suggested that potassium secretion by distal segments of the nephron was not directly affected by vanadate but would vary with changes in sodium concentration in the tubular fluid. The rate of potassium excretion may be affected by various

factors, primarily by varying the rate of secretion. Nevertheless, the possible direct mechanism induced the increase in fractional excretion of potassium in both groups may be caused by the inhibition of H^+-K^+-ATPase in distal nephron segments.

The H^+-K^+-ATPase which belongs to the E_1-E_2 type of ATPase has been reported to have properties similar to gastic H^+-K^+-ATPase and to be inhibited by vanadate and omeprazole (Docet and Marsy, 1987; Gary and Narang, 1988). Recent study has reported that omeprazole abolished both proton secretion and potassium absorption in the outer medullary collecting duct of rabbit conditioned to low-potassium diet by inhibition of H^+-K^+-ATPase (Wingo,1989). Consequently, vanadate that is the inhibitor of H^+-K^+-ATPase may inhibit K^+ absorption in distal nephron leading to increase in potassium excretion.

In this present study, we found that urinary acid excretion decreases following with a continuous infusion of hydrochloric acid solution. Therefore, this may enhance severe metabolic acidosis that leads to occur the increase in plasma concentration of potassium (Narins et al., 1985, quoting Oster et al.) Nevertheless, hyperkalemia usually associated with impaired renal potassium excretion. (Stanton and Giebisch, 1982). A rise in the plasma potassium concentration not only affects the kidney directly by

increasing in potassium secretion (Young, 1982) and also stimulates the adrenal gland to secrete aldosterone (Funder et al., 1969; Boyd and Mulrow, 1972) which may also contribute to enhance potassium excretion. The rate of fluid flow through the distal tubule also has shown to be a factor influencing potassium secretion at this site. Under a wide varieties of circumstance, an increase in luminal flow rate results in an elevation of potassium secretion (Kunau et al., 1974). Changes in tubular fluid composition and flow rate have been shown to interfere proximal tubular reabsorption of salt and water lead to abolish proximal reabsorption of potassium because of gradient limitation (Lawrence and Jared, 1982). Therefore, these changes may cause an increase in fractional excretion of potassium. In our results, plasma concentration of potassium rose significantly after vanadate injection in metabolic acidosis group. This may be affected by the result of acidosis that produces the transcellular shifts of potassium. (Narins et al., 1985). Additional, it may be caused by the effect of vanadate on an inhibition of Na^+-K^+-ATPase leading to inhibit K^+ secretion in the basolateral membrane. This effect leads to increase in plasma potassium concentration in extra-cellular fluid.

In present data, plasma concentration and fractional excretion of chloride are significantly increased in metabolic acidosis. Furthermore, a slightly simultaneous increase of plasma concentration, urinary excretion rate and fractional excretion of chloride has been seen in saline control. This phenomenon may be explained that natriuresis and diuresis diminish chloride reabsorption leading to increase in urinary excretion of chloride. A rise in plasma concentration of chloride may be the result of acidosis that induces the reduction of plasma concentration of bicarbonate (Morris, 1969), so that it initiate chloride to shift out of the cell into extracellular fluid.

In this experiment, plasma concentration of bicarbonate fell significantly in metabolic acidosis while the fractional excretion of bicarbonate slightly rose in both groups but not reached the statistically significant level. A fall in plasma concentration of bicarbonate in our data may be effected by acidosis that interferes bicarbonate buffering system. Moreover, it may be mediated by the effects of vanadate induced an increase in bicarbonate excretion as shown in this study which similar to the results from Day et al. (1980) and Westenfelder et al. (1981). The increase in fractional excretion of bicarbonate can explain that it may be caused by the

effect of vanadate on sodium transport (Westenfelder et al., 1981). The reduction of Na reabsorption by vanadate is due to the inhibition of Na^+-K^+-ATPase activity in proximal tubule and leading to diminish hydrogen secretion. The reduction in bicarbonate generation and reabsorption may result in an increase in urinary bicarbonate excretion. Nevertheless, in both normal and acute metabolic acidosis, vanadate may produce a reduction in hydrogen ion secretion and lower bicarbonate reabsorption in proximal tubule resulting in an elevation of urinary bicarbonate excretion (Adler and Fraley, 1985).

It will be observed that urinary excretion rate of ammonium and titratable acid and also net acid excretion were reduced significantly following vanadate injection in acute metabolic acidosis. In saline control group, they trended to decrease but not reached the statistically significant level as shown in Figure 8. Normally, acid excretion is to a large extent in distal nephron event by secreted hydrogen ion that is excreted in the urine in the form of ammonium and titratable acid. The process of hydrogen secretion in distal nephron segments is active and mediated by a proton translocating ATPase (Toto, 1986) and H^+-K^+-ATPase (Wingo, 1989). The decline in urinary excretion rate of ammonium and titratable acid and net acid excretion in this present study

may be due to the effects of vanadate on the reduction in GFR (Adler and Fraley, 1985) and/or directly inhibition of H^+-K^+-ATPase in distal nephron segments (Doucet and Marsy, 1987; Gary and Narang, 1988). So that, the fractional excretion of potassium increased significantly as shown in Figure 5. It is possibility that K reabsorption and H^+ secretion may be linked functionally (Doucet and Marsy, 1987).

A decrease in urinary excretion rate of ammonium may be affected from the increase in plasma concentration of potassium, since hyperkalemia decreases ammonia production and excretion. (Toto, 1986, quoting Tannen and Hulter). Ordinary, the urinary net acid excretion rate is measured as the sum of ammonium and titratable acid less any residual unreabsorpted bicarbonate; therefore, NAE = NH_4 + TA-HCO_3. It is important to note that limitating of buffer availibility can seriously limit net acid excretion sufficiently to result in systemic acidosis (Toto, 1986). In our results, the urinary net acid excretion appeared to be diminished similar to the urinary excretion rate of ammonium and titratable acid. The decline in these effects may result from vanadate induced the decrease in glomerular fitration rate and hyperkalemia, because decreasing renal function is accompanied by diminished renal hydrogen ion secretion (Adler and Fraley, 1985).

Arruda et al. (1981) have demonstrated that vanadate can inhibit urinary acidification in turtle bladder *in vitro* by inhibition of hydrogen pump. Recently, in vitro study, Youmans and Brodsky (1989) have also shown that vanadate at low concentration (45 nM) inhibited E_1-E_2 type hydrogen transporters in turtle bladder. Therefore, vanadate may affect hydrogen ion transport processes in acid secretion. However, in this present data, it is indicated that vanadate reduced urinary acid excretion. The direct mechanism of vanadate on urinary acid excretion is still unclear.